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(54) Title: NUCLEOTIDE SEQUENCE OF THE HAEMOPHILUS INFLUENZAE Rd GENOME, FRAGMENTS THEREOF, AND USES THEREOF			
(57) Abstract  The present invention provides the sequencing of the entire genome of <i>Haemophilus influenzae</i> Rd, SEQ ID NO:1. The present invention further provides the sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use. In addition to the entire genomic sequence, the present invention identifies over 1700 protein encoding fragments of the genome and identifies, by position relative to a unique <i>Not I</i> restriction endonuclease site, any regulatory elements which modulate the expression of the protein encoding fragments of the <i>Haemophilus</i> genome.			

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## Nucleotide Sequence of the *Haemophilus influenzae* Rd Genome, Fragments Thereof, and Uses Thereof

Part of the work performed during development of this invention utilized U.S. Government funds. The government may have certain rights in this invention. NIH-5R01GM48251

### *Field of the Invention*

The present invention relates to the field of molecular biology. The present invention discloses compositions comprising the nucleotide sequence of *Haemophilus influenzae*, fragments thereof and usage in industrial fermentation and pharmaceutical development.

### *Background of the Invention*

The complete genome sequence from a free living cellular organism has never been determined. The first mycobacterium sequence should be completed by 1996, while *E. coli* and *S. cerevisiae* are expected to be completed before 1998. These are being done by random and/or directed sequencing of overlapping cosmid clones. No one has attempted to determine sequences of the order of a megabase or more by a random shotgun approach.

*H. influenzae* is a small (approximately 0.4 x 1 micron) non-motile, non-spore forming, germ-negative bacterium whose only natural host is human. It is a resident of the upper respiratory mucosa of children and adults and causes otitis media and respiratory tract infections mostly in children. The most serious complication is meningitis, which produces neurological sequelae

in up to 50% of affected children. Six *H. influenzae* serotypes (a through f) have been identified based on immunologically distinct capsular polysaccharide antigens. A number of non-typeable strains are also known. Serotype b accounts for the majority of human disease.

5           Interest in the medically important aspects of *H. influenzae* biology has focused particularly on those genes which determine virulence characteristics of the organism. A number of the genes responsible for the capsular polysaccharide have been mapped and sequenced (Kroll *et al.*, *Mol. Microbiol.* 5(6):1549-1560 (1991)). Several outer membrane protein (OMP) genes have  
10           been identified and sequenced (Langford *et al.*, *J. Gen. Microbiol.* 138:155-159 (1992)). The lipoligosaccharide (LOS) component of the outer membrane and the genes of its synthetic pathway are under intensive study (Weiser *et al.*, *J. Bacteriol.* 172:3304-3309 (1990)). While a vaccine has been available since 1984, the study of outer membrane components is motivated to some extent  
15           by the need for improved vaccines. Recently, the catalase gene was characterized and sequenced as a possible virulence-related gene (Bishni *et al.*, in press). Elucidation of the *H. influenzae* genome will enhance the understanding of how *H. influenzae* causes invasive disease and how best to combat infection.

20           *H. influenzae* possesses a highly efficient natural DNA transformation system which has been intensively studied in the non-encapsulated (R), serotype d strain (Kahn and Smith, *J. Membrane Biology* 81:89-103 (1984)). At least 16 transformation-specific genes have been identified and sequenced. Of these, four are regulatory (Redfield, *J. Bacteriol.* 173:5612-5618 (1991),  
25           and Chandler, *Proc. Natl. Acad. Sci. USA* 89:1626-1630 (1992)), at least two are involved in recombination processes (Barouki and Smith, *J. Bacteriol.* 163(2):629-634 (1985)), and at least seven are targeted to the membranes and periplasmic space (Tomb *et al.*, *Gene* 104:1-10 (1991), and Tomb, *Proc. Natl. Acad. Sci. USA* 89:10252-10256 (1992)), where they appear to function as  
30           structural components or in the assembly of the DNA transport machinery. *H. influenzae* Rd transformation shows a number of interesting features including



sequence-specific DNA uptake, rapid uptake of several double-stranded DNA molecules per competent cell into a membrane compartment called the transformasome, linear translocation of a single strand of the donor DNA into the cytoplasm, and synapsis and recombination of the strand with the chromosome by a single-strand displacement mechanism. The *H. influenzae* Rd transformation system is the most thoroughly studied of the gram-negative systems and distinct in a number of ways from the gram-positive systems.

The size of *H. influenzae* Rd genome has been determined by pulsed-field agarose gel electrophoresis of restriction digests to be approximately 1.9 Mb, making its genome approximately 40% the size of *E. coli* (Lee and Smith, *J. Bacteriol.* 170:4402-4405 (1988)). The restriction map of *H. influenzae* is circular (Lee *et al.*, *J. Bacteriol.* 171:3016-3024 (1989), and Redfield and Lee, "*Haemophilus influenzae* Rd", pp. 2110-2112, In O'Brien, S.J. (ed), Genetic Maps: Locus Maps of Complex Genomes, Cold Spring Harbor Press, New York). Various genes have been mapped to restriction fragments by Southern hybridization probing of restriction digest DNA bands. This map will be valuable in verification of the assembly of a complete genome sequence from randomly sequenced fragments. GenBank currently contains about 100 kb of non-redundant *H. influenzae* DNA sequences. About half are from serotype b and half from Rd.

### *Summary of the Invention*

The present invention is based on the sequencing of the *Haemophilus influenzae* Rd genome. The primary nucleotide sequence which was generated is provided in SEQ ID NO:1.

The present invention provides the generated nucleotide sequence of the *Haemophilus influenzae* Rd genome, or a representative fragment thereof, in a form which can be readily used, analyzed, and interpreted by a skilled artisan. In one embodiment, present invention is provided as a contiguous

string of primary sequence information corresponding to the nucleotide sequence depicted in SEQ ID NO:1.

The present invention further provides nucleotide sequences which are at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1.

5           The nucleotide sequence of SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence which is at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1 may be provided in a variety of mediums to facilitate its use. In one application of this embodiment, the sequences of the present invention are recorded on computer readable media.  
10       Such media includes, but is not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media.

15           The present invention further provides systems, particularly computer-based systems which contain the sequence information herein described stored in a data storage means. Such systems are designed to identify commercially important fragments of the *Haemophilus influenzae* Rd genome.

20           Another embodiment of the present invention is directed to isolated fragments of the *Haemophilus influenzae* Rd genome. The fragments of the *Haemophilus influenzae* Rd genome of the present invention include, but are not limited to, fragments which encode peptides, hereinafter open reading frames (ORFs), fragments which modulate the expression of an operably linked ORF, hereinafter expression modulating fragments (EMFs), fragments which mediate the uptake of a linked DNA fragment into a cell, hereinafter uptake modulating fragments (UMFs), and fragments which can be used to  
25       diagnose the presence of *Haemophilus influenzae* Rd in a sample, hereinafter, diagnostic fragments (DFs).

30           Each of the ORF fragments of the *Haemophilus influenzae* Rd genome disclosed in Tables 1(a) and 2, and the EMF found 5' to the ORF, can be used in numerous ways as polynucleotide reagents. The sequences can be used as diagnostic probes or diagnostic amplification primers for the presence of a

specific microbe in a sample, for the production of commercially important pharmaceutical agents, and to selectively control gene expression.

5 The present invention further includes recombinant constructs comprising one or more fragments of the *Haemophilus influenzae* Rd genome of the present invention. The recombinant constructs of the present invention comprise vectors, such as a plasmid or viral vector, into which a fragment of the *Haemophilus influenzae* Rd has been inserted.

10 The present invention further provides host cells containing any one of the isolated fragments of the *Haemophilus influenzae* Rd genome of the present invention. The host cells can be a higher eukaryotic host such as a mammalian cell, a lower eukaryotic cell such as a yeast cell, or can be a procaryotic cell such as a bacterial cell.

15 The present invention is further directed to isolated proteins encoded by the ORFs of the present invention. A variety of methodologies known in the art can be utilized to obtain any one of the proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. In an alternative method, the protein is purified from bacterial cells which naturally produce the protein. Lastly, the proteins of the present invention can alternatively be purified from  
20 cells which have been altered to express the desired protein.

The invention further provides methods of obtaining homologs of the fragments of the *Haemophilus influenzae* Rd genome of the present invention and homologs of the proteins encoded by the ORFs of the present invention. Specifically, by using the nucleotide and amino acid sequences disclosed herein  
25 as a probe or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain homologs.

The invention further provides antibodies which selectively bind one of the proteins of the present invention. Such antibodies include both monoclonal and polyclonal antibodies.

The invention further provides hybridomas which produce the above-described antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

5       The present invention further provides methods of identifying test samples derived from cells which express one of the ORF of the present invention, or homolog thereof. Such methods comprise incubating a test sample with one or more of the antibodies of the present invention, or one or more of the DFs of the present invention, under conditions which allow a skilled artisan to determine if the sample contains the ORF or product  
10       produced therefrom.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the above-described assays.

Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises: (a) a first  
15       container comprising one of the antibodies, or one of the DFs of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of bound antibodies or hybridized DFs.

Using the isolated proteins of the present invention, the present  
20       invention further provides methods of obtaining and identifying agents capable of binding to a protein encoded by one of the ORFs of the present invention. Specifically, such agents include antibodies (described above), peptides, carbohydrates, pharmaceutical agents and the like. Such methods comprise the steps of:

- 25       (a)     contacting an agent with an isolated protein encoded by one of the ORFs of the present invention; and  
         (b)     determining whether the agent binds to said protein.

The complete genomic sequence of *H. influenzae* will be of great value to all laboratories working with this organism and for a variety of commercial  
30       purposes. Many fragments of the *Haemophilus influenzae* Rd genome will be immediately identified by similarity searches against GenBank or protein

databases and will be of immediate value to *Haemophilus* researchers and for immediate commercial value for the production of proteins or to control gene expression. A specific example concerns PHA synthase. It has been reported that polyhydroxybutyrate is present in the membranes of *H. influenzae* Rd and that the amount correlates with the level of competence for transformation. The PHA synthase that synthesizes this polymer has been identified and sequenced in a number of bacteria, none of which are evolutionarily close to *H. influenzae*. This gene has yet to be isolated from *H. influenzae* by use of hybridization probes or PCR techniques. However, the genomic sequence of the present invention allows the identification of the gene by utilizing search means described below.

Developing the methodology and technology for elucidating the entire genomic sequence of bacterial and other small genomes has and will greatly enhance the ability to analyze and understand chromosomal organization. In particular, sequenced genomes will provide the models for developing tools for the analysis of chromosome structure and function, including the ability to identify genes within large segments of genomic DNA, the structure, position, and spacing of regulatory elements, the identification of genes with potential industrial applications, and the ability to do comparative genomic and molecular phylogeny.

### *Description of the Figures*

Figure 1 - restriction map of the *Haemophilus influenzae* Rd genome.

Figure 2 - Block diagram of a computer system 102 that can be used to implement the computer-based systems of present invention.

Figure 3 - A comparison of experimental coverage of up to approximately 4000 random sequence fragments assembled with AutoAssembler (squares) as compared to Lander-Waterman prediction for a 2.5 Mb genome (triangles) and a 1.6 Mb genome (circles) with a 460 bp average sequence length and a 25 bp overlap.

Figure 4 - Data flow and computer programs used to manage, assemble, edit, and annotate the *H. influenzae* genome. Both Macintosh and Unix platforms are used to handle the AB 373 sequence data files (Kerlavage *et al.*, *Proceedings of the Twenty-Sixth Annual Hawaii International Conference on System Sciences*, IEEE Computer Society Press, Washington D.C., 585 (1993)). **Factura** (AB) is a Macintosh program designed for automatic vector sequence removal and end trimming of sequence files. The program **esp** runs on a Macintosh platform and parses the feature data extracted from the sequence files by **Factura** to the Unix based *H. influenzae* relational database. Assembly is accomplished by retrieving a specific set of sequence files and their associated features using **stp**, an X-windows graphical interface and control program which can retrieve sequences from the *H. influenzae* database using user-defined or standard SQL queries. The sequence files were assembled using **TIGR Assembler**, an assembly engine designed at TIGR for rapid and accurate assembly of thousands of sequence fragments. **TIGR Editor** is a graphical interface which can parse the aligned sequence files from **TIGR Assembler** output and display the alignment and associated electropherograms for contig editing. Identification of putative coding regions was performed with **Genemark** (Borodovsky and McIninch, *Computers Chem.* 17(2):123 (1993)), a Markov and Bayes modeled program for predicting gene locations, and trained on a *H. influenzae* sequence data set. Peptide searches were performed against the three reading frames of each **Genemark** predicted coding region using **blaze** (Brutlag *et al.*, *Computers Chem.* 17:203 (1993)) run on a Maspar MP-2 massively parallel computer with 4096 microprocessors. Results from each frame were combined into a single output file by **mbldt**. Optimal protein alignments were obtained using the program **praze** which extends alignments across potential frameshifts. The output was inspected using a custom graphic viewing program, **gbyob**, that interacts directly with the *H. influenzae* database. The alignments were further used to identify potential frameshift errors and were targeted for additional editing.

Figure 5 - A circular representation of the *H. influenzae* Rd chromosome illustrating the location of each predicted coding region containing a database match as well as selected global features of the genome. **Outer perimeter:** The location of the unique *NotI* restriction site (designated as nucleotide 1), the *RsrII* sites, and the *SmaI* sites. **Outer concentric circle:** The location of each identified coding region for which a gene identification was made. Each coding region location is coded as to role according to the color code in Fig. 6. **Second concentric circle:** Regions of high G/C content (> 42%, red; > 40%, blue) and high A/T content (> 66%, black; > 64%, green). High G/C content regions are specifically associated with the 6 ribosomal operons and the mu-like prophage. **Third concentric circle:** Coverage by lambda clones (blue). Over 300 lambda clones were sequenced from each end to confirm the overall structure of the genome and identify the 6 ribosomal operons. **Fourth concentric circle:** The locations of the 6 ribosomal operons (green), the tRNAs (black) and the cryptic mu-like prophage (blue). **Fifth concentric circle:** Simple tandem repeats. The locations of the following repeats are shown: CTGGCT, GTCT, ATT, AATGGC, TTGA, TTGG, TTTA, TTATC, TGAC, TCGTC, AACC, TTGC, CAAT, CCAA. The putative origin of replication is illustrated by the outward pointing arrows (green) originating near base 603,000. Two potential termination sequences are shown near the opposite midpoint of the circle (red).

Figures 6(A)-6(D)- Complete map of the *H. influenzae* Rd genome. Predicted coding regions are shown on each strand. rRNA and tRNA genes are shown as lines and triangles, respectively. Genes are color-coded by role category as described in the legend. GeneID numbers correspond to those in Tables 1(a), 1(b) and 2. Where possible, three-letter designations are also provided.

Figure 7 - A comparison of the region of the *H. influenzae* chromosome containing the 8 genes of the fimbrial gene cluster present in *H.*

5 *influenzae* type b and the same region in *H. influenzae* Rd. The region is flanked by the *pepN* and *purE* genes in both organisms. However in the non-infectious Rd strain the 8 genes of the fimbrial gene cluster have been excised. A 172 bp spacer region is located in this region in the Rd strain and continues to be flanked by the *pepN* and *purE* genes.

Figure 8 - Hydrophobicity analysis of five predicted channel-proteins. The amino acid sequences of five predicted coding regions that do not display homology with known peptide sequences (GenBank release 87), each exhibit multiple hydrophobic domains that are characteristic of channel-forming proteins. The predicted coding region sequences were analyzed by the Kyte-Doolittle algorithm (Kyte and Doolittle, *J. Mol. Biol.* 157:105 (1982)) (with a range of 11 residues) using the GeneWorks software package (Intelligenetics).

### *Detailed Description of the Preferred Embodiments*

15 The present invention is based on the sequencing of the *Haemophilus influenzae* Rd genome. The primary nucleotide sequence which was generated is provided in SEQ ID NO:1. As used herein, the "primary sequence" refers to the nucleotide sequence represented by the IUPAC nomenclature system.

20 The sequence provided in SEQ ID NO:1 is oriented relative to a unique *Not* I restriction endonuclease site found in the *Haemophilus influenzae* Rd genome. A skilled artisan will readily recognize that this start/stop point was chosen for convenience and does not reflect a structural significance.

25 The present invention provides the nucleotide sequence of SEQ ID NO:1, or a representative fragment thereof, in a form which can be readily used, analyzed, and interpreted by a skilled artisan. In one embodiment, the sequence is provided as a contiguous string of primary sequence information corresponding to the nucleotide sequence provided in SEQ ID NO:1.



As used herein, a "representative fragment of the nucleotide sequence depicted in SEQ ID NO:1" refers to any portion of SEQ ID NO:1 which is not presently represented within a publicly available database. Preferred representative fragments of the present invention are *Haemophilus influenzae* open reading frames, expression modulating fragments, uptake modulating fragments, and fragments which can be used to diagnose the presence of *Haemophilus influenzae* Rd in sample. A non-limiting identification of such preferred representative fragments is provided in Tables 1(a) and 2.

The nucleotide sequence information provided in SEQ ID NO:1 was obtained by sequencing the *Haemophilus influenzae* Rd genome using a megabase shotgun sequencing method. Using three parameters of accuracy discussed in the Examples below, the present inventors have calculated that the sequence in SEQ ID NO:1 has a maximum accuracy of 99.98%. Thus, the nucleotide sequence provided in SEQ ID NO:1 is a highly accurate, although not necessarily a 100% perfect, representation of the nucleotide sequence of the *Haemophilus influenzae* Rd genome.

As discussed in detail below, using the information provided in SEQ ID NO:1 and in Tables 1(a) and 2 together with routine cloning and sequencing methods, one of ordinary skill in the art will be able to clone and sequence all "representative fragments" of interest including open reading frames (ORFs) encoding a large variety of *Haemophilus influenzae* proteins. In very rare instances, this may reveal a nucleotide sequence error present in the nucleotide sequence disclosed in SEQ ID NO: 1. Thus, once the present invention is made available (i.e., once the information in SEQ ID NO:1 and Tables 1(a) and 2 have been made available), resolving a rare sequencing error in SEQ ID NO:1 will be well within the skill of the art. Nucleotide sequence editing software is publicly available. For example, Applied Biosystem's (AB) AutoAssembler™ can be used as an aid during visual inspection of nucleotide sequences.

Even if all of the very rare sequencing errors in SEQ ID NO:1 were corrected, the resulting nucleotide sequence would still be at least 99.9% identical to the nucleotide sequence in SEQ ID NO:1.

5 The nucleotide sequences of the genomes from different strains of *Haemophilus influenzae* differ slightly. However, the nucleotide sequence of the genomes of all *Haemophilus influenzae* strains will be at least 99.9% identical to the nucleotide sequence provided in SEQ ID NO:1.

10 Thus, the present invention further provides nucleotide sequences which are at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1 in a form which can be readily used, analyzed and interpreted by the skilled artisan. Methods for determining whether a nucleotide sequence is at least 99.9% identical to the nucleotide sequence of SEQ ID NO:1 are routine and readily available to the skilled artisan. For example, the well known *fasta* algorithm (Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444  
15 (1988)) can be used to generate the percent identity of nucleotide sequences.

### ***Computer Related Embodiments***

20 The nucleotide sequence provided in SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 may be "provided" in a variety of mediums to facilitate use thereof. As used herein, provided refers to a manufacture, other than an isolated nucleic acid molecule, which contains a nucleotide sequence of the present invention, i.e., the nucleotide sequence provided in SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NO:1. Such a manufacture provides the *Haemophilus influenzae* Rd genome  
25 or a subset thereof (e.g., a *Haemophilus Influenzae* Rd open reading frame (ORF)) in a form which allows a skilled artisan to examine the manufacture using means not directly applicable to examining the *Haemophilus influenzae* Rd genome or a subset thereof as it exists in nature or in purified form.

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention.

As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently know methods for recording information on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of dataprocessor structuring formats (e.g. text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing the nucleotide sequence of SEQ ID NO: 1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 in computer readable form, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)) and BLAZE (Brutlag *et al.*, *Comp. Chem.* 17:203-207 (1993)) search algorithms on a Sybase system was used to identify open reading frames (ORFs) within the *Haemophilus influenzae* Rd genome which contain homology to ORFs or proteins from other organisms. Such ORFs are protein encoding fragments within the *Haemophilus influenzae* Rd genome and are useful in producing commercially important proteins such as enzymes used in fermentation reactions and in the production of commercially useful metabolites.

The present invention further provides systems, particularly computer-based systems, which contain the sequence information described herein. Such systems are designed to identify commercially important fragments of the *Haemophilus influenzae* Rd genome.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based system are suitable for use in the present invention.

As stated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory which can store nucleotide sequence

information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention.

5 As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the *Haemophilus influenzae* Rd genome which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a  
10 variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software includes, but is not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBIA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software  
15 packages for conducting homology searches can be adapted for use in the present computer-based systems.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled  
20 artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that searches for commercially important fragments of the *Haemophilus influenzae* Rd genome, such as sequence fragments involved in  
25 gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target  
30 motifs known in the art. Protein target motifs include, but are not limited to, enzymic active sites and signal sequences. Nucleic acid target motifs include,

but are not limited to, promoter sequences, hairpin structures and inducible expression elements (protein binding sequences).

5 A variety of structural formats for the input and output means can be used to input and output the information in the computer-based systems of the present invention. A preferred format for an output means ranks fragments of the *Haemophilus influenzae* Rd genome possessing varying degrees of homology to the target sequence or target motif. Such presentation provides a skilled artisan with a ranking of sequences which contain various amounts of the target sequence or target motif and identifies the degree of homology  
10 contained in the identified fragment.

A variety of comparing means can be used to compare a target sequence or target motif with the data storage means to identify sequence fragments of the *Haemophilus influenzae* Rd genome. In the present examples, implementing software which implement the BLAST and BLAZE  
15 algorithms (Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990)) was used to identify open reading frames within the *Haemophilus influenzae* Rd genome. A skilled artisan can readily recognize that any one of the publicly available homology search programs can be used as the search means for the computer-based systems of the present invention.

20 One application of this embodiment is provided in Figure 2. Figure 2 provides a block diagram of a computer system 102 that can be used to implement the present invention. The computer system 102 includes a processor 106 connected to a bus 104. Also connected to the bus 104 are a main memory 108 (preferably implemented as random access memory, RAM)  
25 and a variety of secondary storage devices 110, such as a hard drive 112 and a removable medium storage device 114. The removable medium storage device 114 may represent, for example, a floppy disk drive, a CD-ROM drive, a magnetic tape drive, etc. A removable storage medium 116 (such as a floppy disk, a compact disk, a magnetic tape, etc.) containing control logic  
30 and/or data recorded therein may be inserted into the removable medium storage device 114. The computer system 102 includes appropriate software

for reading the control logic and/or the data from the removable medium storage device 114 once inserted in the removable medium storage device 114.

5 A nucleotide sequence of the present invention may be stored in a well known manner in the main memory 108, any of the secondary storage devices 110, and/or a removable storage medium 116. Software for accessing and processing the genomic sequence (such as search tools, comparing tools, etc.) reside in main memory 108 during execution.

### ***Biochemical Embodiments***

10 Another embodiment of the present invention is directed to isolated fragments of the *Haemophilus influenzae* Rd genome. The fragments of the *Haemophilus influenzae* Rd genome of the present invention include, but are not limited to fragments which encode peptides, hereinafter open reading frames (ORFs), fragments which modulate the expression of an operably linked ORF, hereinafter expression modulating fragments (EMFs), fragments  
15 which mediate the uptake of a linked DNA fragment into a cell, hereinafter uptake modulating fragments (UMFs), and fragments which can be used to diagnose the presence of *Haemophilus influenzae* Rd in a sample, hereinafter diagnostic fragments (DFs).

20 As used herein, an "isolated nucleic acid molecule" or an "isolated fragment of the *Haemophilus influenzae* Rd genome" refers to a nucleic acid molecule possessing a specific nucleotide sequence which has been subjected to purification means to reduce, from the composition, the number of compounds which are normally associated with the composition. A variety of purification means can be used to generate the isolated fragments of the  
25 present invention. These include, but are not limited to methods which separate constituents of a solution based on charge, solubility, or size.

In one embodiment, *Haemophilus influenzae* Rd DNA can be mechanically sheared to produce fragments of 15-20 kb in length. These fragments can then be used to generate an *Haemophilus influenzae* Rd library

by inserting them into labda clones as described in the Examples below. Primers flanking, for example, an ORF provided in Table 1(a) can then be generated using nucleotide sequence information provided in SEQ ID NO:1. PCR cloning can then be used to isolate the ORF from the lambda DNA library. PCR cloning is well known in the art. Thus, given the availability of SEQ ID NO:1, Table 1(a) and Table 2, it would be routine to isolate any ORF or other nucleic acid fragment of the present invention.

The isolated nucleic acid molecules of the present invention include, but are not limited to single stranded and double stranded DNA, and single stranded RNA.

As used herein, an "open reading frame," ORF, means a series of triplets coding for amino acids without any termination codons and is a sequence translatable into protein. Tables 1a, 1b and 2 identify ORFs in the *Haemophilus influenzae* Rd genome. In particular, Table 1a indicates the location of ORFs within the *Haemophilus influenzae* genome which encode the recited protein based on homology matching with protein sequences from the organism appearing in parentheses (see the fourth column of Table 1(a)).

The first column of Table 1(a) provides the "GeneID" of a particular ORF. This information is useful for two reasons. First, the complete map of the *Haemophilus influenzae* Rd genome provided in Figures 6(A)-6(D) refers to the ORFs according to their GeneID numbers. Second, Table 1(b) uses the GeneID numbers to indicate which ORFs were provided previously in a public database.

The second and third columns in Table 1(a) indicate an ORFs position in the nucleotide sequence provided in SEQ ID NO:1. One of ordinary skill will recognize that ORFs may be oriented in opposite directions in the *Haemophilus influenzae* genome. This is reflected in columns 2 and 3.

The fifth column of Table 1(a) indicates the percent identity of the protein encoded for by an ORF to the corresponding protein from the organism appearing in parentheses in the fourth column.



5 The sixth column of Table 1(a) indicates the percent similarity of the protein encoded for by an ORF to the corresponding protein from the organism appearing in parentheses in the fourth column. The concepts of percent identity and percent similarity of two polypeptide sequences is well understood  
10 in the art. For example, two polypeptides 10 amino acids in length which differ at three amino acid positions (e.g., at positions 1, 3 and 5) are said to have a percent identity of 70%. However, the same two polypeptides would be deemed to have a percent similarity of 80% if, for example at position 5, the amino acids moieties, although not identical, were "similar" (i.e.,  
15 possessed similar biochemical characteristics).

The seventh column in Table 1(a) indicates the length of the amino acid homology match.

Table 2 provides ORFs of the *Haemophilus influenzae* Rd genome which encode polypeptide sequences which did not elicit a "homology match" with a known protein sequence from another organism. Further details concerning the algorithms and criteria used for homology searches are provided in the Examples below.

20 A skilled artisan can readily identify ORFs in the *Haemophilus influenzae* Rd genome other than those listed in Tables 1(a), 1(b) and 2, such as ORFs which are overlapping or encoded by the opposite strand of an identified ORF in addition to those ascertainable using the computer-based systems of the present invention.

As used herein, an "expression modulating fragment," EMF, means a series of nucleotide molecules which modulates the expression of an operably  
25 linked ORF or EMF.

As used herein, a sequence is said to "modulate the expression of an operably linked sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs  
30 are fragments which induce the expression of an operably linked ORF in response to a specific regulatory factor or physiological event. A review of

known EMFs from *Haemophilus* are described by (Tomb *et al. Gene* 104:1-10 (1991), Chandler, M. S., *Proc. Natl. Acad. Sci. USA* 89:1626-1630 (1992).

EMF sequences can be identified within the *Haemophilus influenzae* Rd genome by their proximity to the ORFs provided in Tables 1(a), 1(b) and 2. An intergenic segment, or a fragment of the intergenic segment, from about 10 to 200 nucleotides in length, taken 5' from any one of the ORFs of Tables 1(a), 1(b), or 2 will modulate the expression of an operably linked 3' ORF in a fashion similar to that found with the naturally linked ORF sequence. As used herein, an "intergenic segment" refers to the fragments of the *Haemophilus* genome which are between two ORF(s) herein described. Alternatively, EMFs can be identified using known EMFs as a target sequence or target motif in the computer-based systems of the present invention.

The presence and activity of an EMF can be confirmed using an EMF trap vector. An EMF trap vector contains a cloning site 5' to a marker sequence. A marker sequence encodes an identifiable phenotype, such as antibiotic resistance or a complementing nutrition auxotrophic factor, which can be identified or assayed when the EMF trap vector is placed within an appropriate host under appropriate conditions. As described above, a EMF will modulate the expression of an operably linked marker sequence. A more detailed discussion of various marker sequences is provided below.

A sequence which is suspected as being a EMF is cloned in all three reading frames in one or more restriction sites upstream from the marker sequence in the EMF trap vector. The vector is then transformed into an appropriate host using known procedures and the phenotype of the transformed host is examined under appropriate conditions. As described above, an EMF will modulate the expression of an operably linked marker sequence.

As used herein, an "uptake modulating fragment," UMF, means a series of nucleotide molecules which mediate the uptake of a linked DNA fragment into a cell. UMFs can be readily identified using known UMFs as a target sequence or target motif with the computer-based systems described above.

The presence and activity of a UMF can be confirmed by attaching the suspected UMF to a marker sequence. The resulting nucleic acid molecule is then incubated with an appropriate host under appropriate conditions and the uptake of the marker sequence is determined. As described above, a UMF will increase the frequency of uptake of a linked marker sequence. A review of DNA uptake in *Haemophilus* is provided by Goodgall, S.H., *et al.*, *J. Bact.* 172:5924-5928 (1990).

As used herein, a "diagnostic fragment," DF, means a series of nucleotide molecules which selectively hybridize to *Haemophilus influenzae* sequences. DFs can be readily identified by identifying unique sequences within the *Haemophilus influenzae* Rd genome, or by generating and testing probes or amplification primers consisting of the DF sequence in an appropriate diagnostic format which determines amplification or hybridization selectivity.

The sequences falling within the scope of the present invention are not limited to the specific sequences herein described, but also include allelic and species variations thereof. Allelic and species variations can be routinely determined by comparing the sequence provided in SEQ ID NO:1, a representative fragment thereof, or a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 with a sequence from another isolate of the same species. Furthermore, to accommodate codon variability, the invention includes nucleic acid molecules coding for the same amino acid sequences as do the specific ORFs disclosed herein. In other words, in the coding region of an ORF, substitution of one codon for another which encodes the same amino acid is expressly contemplated.

Any specific sequence disclosed herein can be readily screened for errors by resequencing a particular fragment, such as an ORF, in both directions (i.e., sequence both strands). Alternatively, error screening can be performed by sequencing corresponding polynucleotides of *Haemophilus influenzae* origin isolated by using part or all of the fragments in question as a probe or primer.

Each of the ORFs of the *Haemophilus influenzae* Rd genome disclosed in Tables 1(a), 1(b) and 2, and the EMF found 5' to the ORF, can be used in numerous ways as polynucleotide reagents. The sequences can be used as diagnostic probes or diagnostic amplification primers to detect the presence of a specific microbe, such as *Haemophilus influenzae* RD, in a sample. This is especially the case with the fragments or ORFs of Table 2, which will be highly selective for *Haemophilus influenzae*.

In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to DNA or RNA. Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, *Nucl. Acids Res.* 6:3073 (1979); Cooney *et al.*, *Science* 241:456 (1988); and Dervan *et al.*, *Science* 251:1360 (1991)) or to the mRNA itself (antisense - Okano, *J. Neurochem.* 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988)).

Triple helix- formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide.

The present invention further provides recombinant constructs comprising one or more fragments of the *Haemophilus influenzae* Rd genome of the present invention. The recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a fragment of the *Haemophilus influenzae* Rd has been inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present invention, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. For vectors comprising the EMFs and UMFs of the present invention, the vector may further comprise a marker sequence or heterologous ORF operably linked to the EMF or UMF. Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia).

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P<sub>R</sub>, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

The present invention further provides host cells containing any one of the isolated fragments of the *Haemophilus influenzae* Rd genome of the present invention, wherein the fragment has been introduced into the host cell using known transformation methods. The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a procaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. *et al.*, *Basic Methods in Molecular Biology* (1986)).

The host cells containing one of the fragments of the *Haemophilus influenzae* Rd genome of the present invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

The present invention further provides isolated polypeptides encoded by the nucleic acid fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (e.g., an ORF) by nucleotide sequence but, due to the degeneracy of the Genetic Code, encode an identical polypeptide sequence. Preferred nucleic acid fragments of the present invention are the ORFs depicted in Table 1(a) which encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. This is particularly useful in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known methods for isolating polypeptides and proteins in

order to obtain one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immuno-affinity chromatography.

5           The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not  
10           produce or which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention.

15           Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, Cv-1 cell, COS cells, and Sf9 cells, as well as prokaryotic host such as *E. coli* and *B. subtilis*. The most preferred cells are those which do not normally express the particular polypeptide or  
20           protein or which expresses the polypeptide or protein at low natural level.

          "Recombinant," as used herein, means that a polypeptide or protein is derived from recombinant (e.g., microbial or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (e.g., yeast) expression systems. As a product, "recombinant  
25           microbial" defines a polypeptide or protein essentially free of native endogenous substances and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, e.g., *E. coli*, will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern different from that expressed in  
30           mammalian cells.

"Nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. Generally, DNA segments encoding the polypeptides and proteins provided by this invention are assembled from fragments of the *Haemophilus influenzae* Rd genome and short oligonucleotide linkers, or from  
5 a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon.

"Recombinant expression vehicle or vector" refers to a plasmid or phage or virus or vector, for expressing a polypeptide from a DNA (RNA) sequence.  
10 The expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. Structural  
15 units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may or may not be subsequently cleaved from the  
20 expressed recombinant protein to provide a final product.

"Recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extra chromosomally. The cells can be prokaryotic or eukaryotic. Recombinant expression systems as defined herein  
25 will express heterologous polypeptides or proteins upon induction of the regulatory elements linked to the DNA segment or synthetic gene to be expressed.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation  
30 systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and



expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, *et al.*, in *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, New York (1989), the disclosure of which is hereby incorporated by reference.

5           Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from  
10           operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the  
15           periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

          Useful expression vectors for bacterial use are constructed by inserting a  
20           structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable  
25           prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may, also be employed as a matter of choice.

          As a representative but nonlimiting example, useful expression vectors for  
30           bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic

elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM 1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

5

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is derepressed by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

10

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell* 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

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Recombinant polypeptides and proteins produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any

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convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

5 The present invention further includes isolated polypeptides, proteins and nucleic acid molecules which are substantially equivalent to those herein described. As used herein, substantially equivalent can refer both to nucleic acid and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between reference and subject sequences. For purposes of the present invention, sequences having equivalent biological activity, and equivalent expression characteristics are considered substantially equivalent. For purposes of determining equivalence, truncation of the mature sequence should be disregarded.

15 The invention further provides methods of obtaining homologs from other strains of *Haemophilus influenzae*, of the fragments of the *Haemophilus influenzae* Rd genome of the present invention and homologs of the proteins encoded by the ORFs of the present invention. As used herein, a sequence or protein of *Haemophilus influenzae* is defined as a homolog of a fragment of the *Haemophilus influenzae* Rd genome or a protein encoded by one of the ORFs of the present invention, if it shares significant homology to one of the fragments of the *Haemophilus influenzae* Rd genome of the present invention or a protein encoded by one of the ORFs of the present invention. Specifically, by using the sequence disclosed herein as a probe or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain homologs.

25 As used herein, two nucleic acid molecules or proteins are said to "share significant homology" if the two contain regions which process greater than 85% sequence (amino acid or nucleic acid) homology.

30 Region specific primers or probes derived from the nucleotide sequence provided in SEQ ID NO:1 or from a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 can be used to prime DNA synthesis and PCR amplification, as well as to identify colonies containing cloned DNA encoding

a homolog using known methods (Innis *et al.*, *PCR Protocols*, Academic Press, San Diego, CA (1990)).

When using primers derived from SEQ ID NO:1 or from a nucleotide sequence at least 99.9% identical to SEQ ID NO:1, one skilled in the art will recognize that by employing high stringency conditions (e.g., annealing at 50-60°C) only sequences which are greater than 75% homologous to the primer will be amplified. By employing lower stringency conditions (e.g., annealing at 35-37°C), sequences which are greater than 40-50% homologous to the primer will also be amplified.

When using DNA probes derived from SEQ ID NO:1 or from a nucleotide sequence at least 99.9% identical to SEQ ID NO:1 for colony/plaque hybridization, one skilled in the art will recognize that by employing high stringency conditions (e.g., hybridizing at 50-65°C in 5X SSPC and 50% formamide, and washing at 50-65°C in 0.5X SSPC), sequences having regions which are greater than 90% homologous to the probe can be obtained, and that by employing lower stringency conditions (e.g., hybridizing at 35-37°C in 5X SSPC and 40-45% formamide, and washing at 42°C in SSPC), sequences having regions which are greater than 35-45% homologous to the probe will be obtained.

Any organism can be used as the source for homologs of the present invention so long as the organism naturally expresses such a protein or contains genes encoding the same. The most preferred organism for isolating homologs are bacterias which are closely related to *Haemophilus influenzae* Rd.

### *Uses for the Compositions of the Invention*

Each ORF provided in Table 1(a) was assigned to one of 102 biological role categories adapted from Riley, M., *Microbiology Reviews* 57(4):862 (1993)). This allows the skilled artisan to determine a use for each identified coding sequence. Tables 1(a) further provides an identification of the type of polypeptide which is encoded for by each ORF. As a result, one skilled in the art can use the polypeptides of the present invention for commercial,

therapeutic and industrial purposes consistent with the type of putative identification of the polypeptide.

Such identifications permit one skilled in the art to use the *Haemophilus influenzae* ORFs in a manner similar to the known type of sequences for which the identification is made; for example, to ferment a particular sugar source or to produce a particular metabolite. (For a review of enzymes used within the commercial industry, see *Biochemical Engineering and Biotechnology Handbook* 2nd, eds. Macmillan Publ. Ltd., NY (1991) and *Biocatalysts in Organic Syntheses*, ed. J. Tramper *et al.*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985)).

### 1. *Biosynthetic Enzymes*

Open reading frames encoding proteins involved in mediating the catalytic reactions involved in intermediary and macromolecular metabolism, the biosynthesis of small molecules, cellular processes and other functions includes enzymes involved in the degradation of the intermediary products of metabolism, enzymes involved in central intermediary metabolism, enzymes involved in respiration, both aerobic and anaerobic, enzymes involved in fermentation, enzymes involved in ATP proton motor force conversion, enzymes involved in broad regulatory function, enzymes involved in amino acid synthesis, enzymes involved in nucleotide synthesis, enzymes involved in cofactor and vitamin synthesis, can be used for industrial biosynthesis. The various metabolic pathways present in *Haemophilus* can be identified based on absolute nutritional requirements as well as by examining the various enzymes identified in Table 1(a).

Identified within the category of intermediary metabolism, a number of the proteins encoded by the identified ORFs in Tables 1(a) are particularly involved in the degradation of intermediary metabolites as well as non-macromolecular metabolism. Some of the enzymes identified include amylases, glucose oxidases, and catalase.

Proteolytic enzymes are another class of commercially important enzymes. Proteolytic enzymes find use in a number of industrial processes including the processing of flax and other vegetable fibers, in the extraction, clarification and depectinization of fruit juices, in the extraction of vegetables' oil and in the maceration of fruits and vegetables to give unicellular fruits. A detailed review of the proteolytic enzymes used in the food industry is provided by Rombouts *et al.*, *Symbiosis* 21:79 (1986) and Voragen *et al.* in *Biocatalyst in Agricultural Biotechnology*, edited J.R. Whitaker *et al.*, *American Chemical Society Symposium Series* 389:93 (1989)).

The metabolism of glucose, galactose, fructose and xylose are important parts of the primary metabolism of *Haemophilus*. Enzymes involved in the degradation of these sugars can be used in industrial fermentation. Some of the important sugar transforming enzymes, from a commercial viewpoint, include sugar isomerases such as glucose isomerase. Other metabolic enzymes have found commercial use such as glucose oxidases which produces ketogulonic acid (KGA). KGA is an intermediate in the commercial production of ascorbic acid using the Reichstein's procedure (*see* Krueger *et al.*, *Biotechnology* 6(A), Rhine, H.J. *et al.*, eds., Verlag Press, Weinheim, Germany (1984)).

Glucose oxidase (GOD) is commercially available and has been used in purified form as well as in an immobilized form for the deoxygenation of beer. *See* Hartmeir *et al.*, *Biotechnology Letters* 1:21 (1979). The most important application of GOD is the industrial scale fermentation of gluconic acid. Market for gluconic acids which are used in the detergent, textile, leather, photographic, pharmaceutical, food, feed and concrete industry (*see* Bigelis in *Gene Manipulations and Fungi*, Benett, J.W. *et al.*, eds., Academic Press, New York (1985), p. 357). In addition to industrial applications, GOD has found applications in medicine for quantitative determination of glucose in body fluids recently in biotechnology for analyzing syrups from starch and cellulose hydrosylates. *See* Owusu *et al.*, *Biochem. et Biophysica. Acta.* 872:83 (1986).

The main sweetener used in the world today is sugar which comes from sugar beets and sugar cane. In the field of industrial enzymes, the glucose isomerase process shows the largest expansion in the market today. Initially, soluble enzymes were used and later immobilized enzymes were developed  
5 (Krueger *et al.*, *Biotechnology, The Textbook of Industrial Microbiology*, Sinauer Associated Incorporated, Sunderland, Massachusetts (1990)). Today, the use of glucose-produced high fructose syrups is by far the largest industrial business using immobilized enzymes. A review of the industrial use of these enzymes is provided by Jorgensen, *Starch* 40:307 (1988).

10 Proteinases, such as alkaline serine proteinases, are used as detergent additives and thus represent one of the largest volumes of microbial enzymes used in the industrial sector. Because of their industrial importance, there is a large body of published and unpublished information regarding the use of these enzymes in industrial processes. (See Faultman *et al.*, *Acid Proteases*  
15 *Structure Function and Biology*, Tang, J., ed., Plenum Press, New York (1977) and Godfrey *et al.*, *Industrial Enzymes*, MacMillan Publishers, Surrey, UK (1983) and Hepner *et al.*, *Report Industrial Enzymes by 1990*, Hel Hepner & Associates, London (1986)).

Another class of commercially usable proteins of the present invention  
20 are the microbial lipases identified in Table 1 (see Macrae *et al.*, *Philosophical Transactions of the Chiral Society of London* 310:227 (1985) and Poserke, *Journal of the American Oil Chemist Society* 61:1758 (1984). A major use of lipases is in the fat and oil industry for the production of neutral glycerides using lipase catalyzed inter-esterification of readily available triglycerides.  
25 Application of lipases include the use as a detergent additive to facilitate the removal of fats from fabrics in the course of the washing procedures.

The use of enzymes, and in particular microbial enzymes, as catalyst for key steps in the synthesis of complex organic molecules is gaining popularity at a great rate. One area of great interest is the preparation of chiral  
30 intermediates. Preparation of chiral intermediates is of interest to a wide range of synthetic chemists particularly those scientists involved with the preparation

of new pharmaceuticals, agrochemicals, fragrances and flavors. (See Davies *et al.*, *Recent Advances in the Generation of Chiral Intermediates Using Enzymes*, CRC Press, Boca Raton, Florida (1990)). The following reactions catalyzed by enzymes are of interest to organic chemists: hydrolysis of carboxylic acid esters, phosphate esters, amides and nitriles, esterification reactions, trans-esterification reactions, synthesis of amides, reduction of alkanones and oxoalkanates, oxidation of alcohols to carbonyl compounds, oxidation of sulfides to sulfoxides, and carbon bond forming reactions such as the aldol reaction. When considering the use of an enzyme encoded by one of the ORFs of the present invention for biotransformation and organic synthesis it is sometimes necessary to consider the respective advantages and disadvantages of using a microorganism as opposed to an isolated enzyme. Pros and cons of using a whole cell system on the one hand or an isolated partially purified enzyme on the other hand, has been described in detail by Bud *et al.*, *Chemistry in Britain* (1987), p. 127.

Amino transferases, enzymes involved in the biosynthesis and metabolism of amino acids, are useful in the catalytic production of amino acids. The advantages of using microbial based enzyme systems is that the amino transferase enzymes catalyze the stereo-selective synthesis of only *l*-amino acids and generally possess uniformly high catalytic rates. A description of the use of amino transferases for amino acid production is provided by Roselle-David, *Methods of Enzymology* 136:479 (1987).

Another category of useful proteins encoded by the ORFs of the present invention include enzymes involved in nucleic acid synthesis, repair, and recombination. A variety of commercially important enzymes have previously been isolated from members of *Haemophilus* sp. These include the Hinc II, Hind III, and Hinf I restriction endonucleases. Table 1(a) identifies a wide array of enzymes, such as restriction enzymes, ligases, gyrases and methylases, which have immediate use in the biotechnology industry.

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## 2. Generation of Antibodies



As described here, the proteins of the present invention, as well as homologs thereof, can be used in a variety of procedures and methods known in the art which are currently applied to other proteins. The proteins of the present invention can further be used to generate an antibody which selectively binds the protein. Such antibodies can be either monoclonal or polyclonal antibodies, as well as fragments of these antibodies, and humanized forms.

The invention further provides antibodies which selectively bind to one of the proteins of the present invention and hybridomas which produce these antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing polyclonal and monoclonal antibodies as well as hybridomas capable of producing the desired antibody are well known in the art (Campbell, A.M., *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth *et al.*, *J. Immunol. Methods* 35:1-21 (1980); Kohler and Milstein, *Nature* 256:495-497 (1975)), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* 4:72 (1983); Cole *et al.*, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc. (1985), pp. 77-96).

Any animal (mouse, rabbit, etc.) which is known to produce antibodies can be immunized with the pseudogene polypeptide. Methods for immunization are well known in the art. Such methods include subcutaneous or intraperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of the protein encoded by the ORF of the present invention used for immunization will vary based on the animal which is immunized, the antigenicity of the peptide and the site of injection.

The protein which is used as an immunogen may be modified or administered in an adjuvant in order to increase the protein's antigenicity. Methods of increasing the antigenicity of a protein are well known in the art and include, but are not limited to coupling the antigen with a heterologous

protein (such as globulin or  $\beta$ -galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells.

Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz *et al.*, *Exp. Cell Res.* 175:109-124 (1988)).

Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, A.M., *Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1984)).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to proteins of the present invention.

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

The present invention further provides the above-described antibodies in detectably labelled form. Antibodies can be detectably labelled through the use of radioisotopes, affinity labels (such as biotin, avidin, etc.), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, etc.) fluorescent labels (such as FITC or rhodamine, etc.), paramagnetic atoms, etc. Procedures for accomplishing such labelling are well-known in the art, for example see (Sternberger, L.A. *et al.*, *J. Histochem. Cytochem.* 18:315 (1970); Bayer, E.A. *et al.*, *Meth. Enzym.* 62:308 (1979); Engval, E. *et al.*, *Immunol.* 109:129 (1972); Goding, J.W. *J. Immunol. Meth.* 13:215 (1976)).

The labeled antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays to identify cells or tissues in which a fragment of the *Haemophilus influenzae* Rd genome is expressed.

5 The present invention further provides the above-described antibodies immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir, D.M. *et al.*, "*Handbook of Experimental Immunology*" 4th Ed.,  
10 Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby, W.D. *et al.*, *Meth. Enzym.* 34 Academic Press, N.Y. (1974)). The immobilized antibodies of the present invention can be used for *in vitro*, *in vivo*, and *in situ* assays as well as for immunoaffinity purification of the proteins of the present invention.

### 15 3. *Diagnostic Assays and Kits*

The present invention further provides methods to identify the expression of one of the ORFs of the present invention, or homolog thereof, in a test sample, using one of the DFs or antibodies of the present invention.

20 In detail, such methods comprise incubating a test sample with one or more of the antibodies or one or more of the DFs of the present invention and assaying for binding of the DFs or antibodies to components within the test sample.

25 Conditions for incubating a DF or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the DF or antibody used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the DFs or antibodies of the present invention. Examples of such assays can be found in Chard, T., *An*

5 *Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. *et al.*, *Techniques in Immunocytochemistry*, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., *Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

10 The test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

15 In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention.

20 Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the DFs or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound DF or antibody.

25 In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a  
30 container which will accept the test sample, a container which contains the

antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which contain the reagents used to detect the bound antibody or DF.

5           Types of detection reagents include labelled nucleic acid probes, labelled secondary antibodies, or in the alternative, if the primary antibody is labelled, the enzymatic, or antibody binding reagents which are capable of reacting with the labelled antibody. One skilled in the art will readily recognize that the disclosed DFs and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well  
10           known in the art.

#### 4.       *Screening Assay for Binding Agents*

          Using the isolated proteins of the present invention, the present invention further provides methods of obtaining and identifying agents which bind to a protein encoded by one of the ORFs of the present invention or to  
15           one of the fragments and the *Haemophilus* genome herein described.

          In detail, said method comprises the steps of:

- (a)       contacting an agent with an isolated protein encoded by one of the ORFs of the present invention, or an isolated fragment of the *Haemophilus* genome; and
- 20       (b)       determining whether the agent binds to said protein or said fragment.

          The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or  
25           designed using protein modeling techniques.

          For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention.

Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby *et al.*, Application of Synthetic Peptides: Antisense Peptides," In *Synthetic Peptides, A User's Guide*, W.H. Freeman, NY (1992), pp. 289-307, and Kaspczak *et al.*, *Biochemistry* 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control.

One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix formation by binding to DNA or RNA. Such agents can be based on the classic phosphodiester, ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, *Nucl. Acids Res.* 6:3073 (1979); Cooney *et al.*, *Science* 241:456 (1988); and Dervan *et al.*, *Science* 251: 1360 (1991)) or to the mRNA itself (antisense - Okano, *J. Neurochem.* 56:560 (1991); *Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression*, CRC Press, Boca Raton, FL (1988)). Triple helix- formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA

hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide and other DNA binding agents.

Agents which bind to a protein encoded by one of the ORFs of the present invention can be used as a diagnostic agent, in the control of bacterial infection by modulating the activity of the protein encoded by the ORF. Agents which bind to a protein encoded by one of the ORFs of the present invention can be formulated using known techniques to generate a pharmaceutical composition for use in controlling *Haemophilus* growth and infection.

#### 5. *Vaccine and Pharmaceutical Composition*

The present invention further provides pharmaceutical agents which can be used to modulate the growth of *Haemophilus influenzae*, or another related organism, *in vivo* or *in vitro*. As used herein, a "pharmaceutical agent" is defined as a composition of matter which can be formulated using known techniques to provide a pharmaceutical compositions. As used herein, the "pharmaceutical agents of the present invention" refers the pharmaceutical agents which are derived from the proteins encoded by the ORFs of the present invention or are agents which are identified using the herein described assays.

As used herein, a pharmaceutical agent is said to "modulated the growth of *Haemophilus sp.*, or a related organism, *in vivo* or *in vitro*," when the agent reduces the rate of growth, rate of division, or viability of the organism in question. The pharmaceutical agents of the present invention can modulate the growth of an organism in many fashions, although an understanding of the underlying mechanism of action is not needed to practice the use of the pharmaceutical agents of the present invention. Some agents will modulate the growth by binding to an important protein thus blocking the

biological activity of the protein, while other agents may bind to a component of the outer surface of the organism blocking attachment or rendering the organism more prone to act the bodies nature immune system. Alternatively, the agent may be comprise a protein encoded by one of the ORFs of the present invention and serve as a vaccine. The development and use of a vaccine based on outer membrane components, such as the LPS, are well known in the art.

As used herein, a "related organism" is a broad term which refers to any organism whose growth can be modulated by one of the pharmaceutical agents of the present invention. In general, such an organism will contain a homolog of the protein which is the target of the pharmaceutical agent or the protein used as a vaccine. As such, related organism do not need to be bacterial but may be fungal or viral pathogens.

The pharmaceutical agents and compositions of the present invention may be administered in a convenient manner such as by the oral, topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, they are administered in an amount of at least about 10  $\mu\text{g/kg}$  body weight and in most cases they will be administered in an amount not in excess of about 8 mg/Kg body weight per day. In most cases, the dosage is from about 10  $\mu\text{g/kg}$  to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The agents of the present invention can be used in native form or can be modified to form a chemical derivative. As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, etc. The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, etc. Moieties capable of



mediating such effects are disclosed in *Remington's Pharmaceutical Sciences* (1980).

For example, a change in the immunological character of the functional derivative, such as affinity for a given antibody, is measured by a competitive type immunoassay. Changes in immunomodulation activity are measured by the appropriate assay. Modifications of such protein properties as redox or thermal stability, biological half-life, hydrophobicity, susceptibility to proteolytic degradation or the tendency to aggregate with carriers or into multimers are assayed by methods well known to the ordinarily skilled artisan.

The therapeutic effects of the agents of the present invention may be obtained by providing the agent to a patient by any suitable means (i.e., inhalation, intravenously, intramuscularly, subcutaneously, enterally, or parenterally). It is preferred to administer the agent of the present invention so as to achieve an effective concentration within the blood or tissue in which the growth of the organism is to be controlled.

To achieve an effective blood concentration, the preferred method is to administer the agent by injection. The administration may be by continuous infusion, or by single or multiple injections.

In providing a patient with one of the agents of the present invention, the dosage of the administered agent will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, previous medical history, etc. In general, it is desirable to provide the recipient with a dosage of agent which is in the range of from about 1 pg/kg to 10 mg/kg (body weight of patient), although a lower or higher dosage may be administered. The therapeutically effective dose can be lowered by using combinations of the agents of the present invention or another agent.

As used herein, two or more compounds or agents are said to be administered "in combination" with each other when either (1) the physiological effects of each compound, or (2) the serum concentrations of each compound can be measured at the same time. The composition of the

present invention can be administered concurrently with, prior to, or following the administration of the other agent.

5 The agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to decrease the rate of growth (as defined above) of the target organism.

10 The administration of the agent(s) of the invention may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the agent(s) are provided in advance of any symptoms indicative of the organisms growth. The prophylactic administration of the agent(s) serves to prevent, attenuate, or decrease the rate of onset of any subsequent infection. When provided therapeutically, the agent(s) are provided at (or shortly after) the onset of an indication of infection. The therapeutic administration of the compound(s) serves to attenuate the pathological symptoms of the infection and to increase the rate of recovery.

15 The agents of the present invention are administered to the mammal in a pharmaceutically acceptable form and in a therapeutically effective concentration. A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

20 The agents of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these materials, or their functional derivatives, are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton PA (1980)). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions

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will contain an effective amount of one or more of the agents of the present invention, together with a suitable amount of carrier vehicle.

Additional pharmaceutical methods may be employed to control the duration of action. Control release preparations may be achieved through the use of polymers to complex or absorb one or more of the agents of the present invention. The controlled delivery may be exercised by selecting appropriate macromolecules (for example polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine, sulfate) and the concentration of macromolecules as well as the methods of incorporation in order to control release. Another possible method to control the duration of action by controlled release preparations is to incorporate agents of the present invention into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacrylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* (1980).

The invention further provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the agents of the present invention may be employed in conjunction with other therapeutic compounds.

## 6. *Shot-Gun Approach to Megabase DNA Sequencing*

5 The present invention further provides the first demonstration that a sequence of greater than one megabase can be sequenced using a random shotgun approach. This procedure, described in detail in the examples that follow, has eliminated the up front cost of isolating and ordering overlapping or contiguous subclones prior to the start of the sequencing protocols.

Certain aspects of the present invention are described in greater detail in the non-limiting Examples that follow.

## Examples

### Experimental Design and Methods

#### 1. Shotgun Sequencing Strategy

The overall strategy for a shotgun approach to whole genome sequencing is outlined in Table 3. The theory of shotgun sequencing follows from the Lander and Waterman (Landerman and Waterman, *Genomics* 2: 231 (1988)) application of the equation for the Poisson distribution  $p_x = m^x e^{-m} / x!$ , where  $x$  is the number of occurrences of an event,  $m$  is the mean number of occurrences, and  $p_x$  is the probability that any given base is not sequenced after a certain amount of random sequence has been generated. If  $L$  is the genome length,  $n$  is the number of clone insert ends sequenced, and  $w$  is the sequencing read length, then  $m = nw/L$ , and the probability that no clone originates at any of the  $w$  bases preceding a given base, i.e., the probability that the base is not sequenced, is  $p_0 = e^{-m}$ . Using the fold coverage as the unit for  $m$ , one sees that after 1.8 Mb of sequence has been randomly generated,  $m = 1$ , representing 1X coverage. In this case,  $p_0 = e^{-1} = .37$ , thus approximately 37% is unsequenced. For example, 5X coverage (approximately 9500 clones sequenced from both insert ends and an average sequence read length of 460 bp) yields  $p_0 = e^{-5} = 0.0067$ , or 0.67% unsequenced. The total gap length is  $Le^{-m}$ , and the average gap size is  $L/n$ . 5X coverage would leave about 128 gaps averaging about 100 bp in size. The treatment is essentially that of Lander and Waterman, *Genomics* 2:231 (1988). Table 4 illustrates the coverage for a 1.9 Mb genome with an average fragment size of 460 bp.

## 2. *Random Library Construction*

In order to approximate the random model described above during actual sequencing, a nearly ideal library of cloned genomic fragment is required. The following library construction procedure was developed to achieve this.

*H. influenzae* Rd KW20 DNA was prepared by phenol extraction. A mixture (3.3 ml) containing 600  $\mu$ g DNA, 300 mM sodium acetate, 10 mM Tris-HCl, 1 mM Na-EDTA, 30% glycerol was sonicated (Branson Model 450 Sonicator) at the lowest energy setting for 1 min. at 0° using a 3 mm probe. The DNA was ethanol precipitated and redissolved in 500  $\mu$ l TE buffer. To create blunt-ends, a 100  $\mu$ l aliquot was digested for 10 min at 30° in 200  $\mu$ l BAL31 buffer with 5 units BAL31 nuclease (New England BioLabs). The DNA was phenol-extracted, ethanol-precipitated, redissolved in 100  $\mu$ l TE buffer, electrophoresed on a 1.0% low melting agarose gel, and the 1.6-2.0 kb size fraction was excised, phenol-extracted, and redissolved in 20  $\mu$ l TE buffer. A two-step ligation procedure was used to produce a plasmid library with 97% insert of which >99% were single inserts. The first ligation mixture (50  $\mu$ l) contained 2  $\mu$ g of DNA fragments, 2  $\mu$ g *Sma*I/BAP pUC18 DNA (Pharmacia), and 10 units T4 ligase (GIBCO/BRL), and incubation was at 14° for 4 hr. After phenol extraction and ethanol precipitation, the DNA was dissolved in 20  $\mu$ l TE buffer and electrophoresed on a 1.0% low melting agarose gel. A ladder of ethidium bromide-stained linear bands, identified by size as insert (i), vector (v), v+i, v+2i, v+3i, ... was visualized by 360 nm UV light, and the v+i DNA was excised and recovered in 20  $\mu$ l TE. The v+i DNA was blunt-ended by T4 polymerase treatment for 5 min. at 37° in a reaction mixture (50  $\mu$ l) containing the v+i linears, 500  $\mu$ M each of the 4 dNTP's, and 9 units of T4 polymerase (New England BioLabs) under recommended buffer conditions. After phenol extraction and ethanol precipitation the repaired v+i linears were dissolved in 20  $\mu$ l TE. The final ligation to produce circles was carried out in a 50  $\mu$ l reaction containing 5  $\mu$ l of v+i linears and 5 units of T4 ligase at 14° overnight. After 10 min. at 70° the reaction mixture was stored at -20°.

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This two-stage procedure resulted in a molecularly random collection of single-insert plasmid recombinants with minimal contamination from double-insert chimeras (<1%) or free vector (<3%). Since deviation from randomness is most likely to occur during cloning, *E. coli* host cells deficient in all recombination and restriction functions (A. Greener, *Strategies* 3 (1):5 (1990)) were used to prevent rearrangements, deletions, and loss of clones by restriction. Transformed cells were plated directly on antibiotic diffusion plates to avoid the usual broth recovery phase which allows multiplication and selection of the most rapidly growing cells. Plating occurred as follows:

A 100  $\mu$ l aliquot of Epicurian Coli SURE II Supercompetent Cells (Stratagene 200152) was thawed on ice and transferred to a chilled Falcon 2059 tube on ice. A 1.7  $\mu$ l aliquot of 1.42 M  $\beta$ -mercaptoethanol was added to the aliquot of cells to a final concentration of 25 mM. Cells were incubated on ice for 10 min. A 1  $\mu$ l aliquot of the final ligation was added to the cells and incubated on ice for 30 min. The cells were heat pulsed for 30 sec. at 42° and placed back on ice for 2 min. The outgrowth period in liquid culture was eliminated from this protocol in order to minimize the preferential growth of any given transformed cell. Instead the transformation were plated directly on a nutrient rich SOB plate containing a 5 ml bottom layer of SOB agar (1.5% SOB agar: 20 g tryptone, 5 g yeast extract, 0.5 g NaCl, 1.5% Difco Agar/L). The 5 ml bottom layer is supplemented with 0.4 ml ampicillin (50 mg/ml)/100 ml SOB agar. The 15 ml top layer of SOB agar is supplemented with 1 ml X-Gal (2%), 1 ml  $MgCl_2$  (1 M), and 1 ml  $MgSO_4$ /100 ml SOB agar. The 15 ml top layer was poured just prior to plating. Our titer was approximately 100 colonies/10  $\mu$ l aliquot of transformation.

All colonies were picked for template preparation regardless of size. Only clones lost due to "poison" DNA or deleterious gene products would be deleted from the library, resulting in a slight increase in gap number over that expected.

In order to evaluate the quality of the *H. influenzae* library, sequence data were obtained from approximately 4000 templates using the M13-21 primer. The random sequence fragments were assembled using the AutoAssembler™ software (Applied Biosystems division of Perkin-Elmer (AB)) after obtaining 1300, 1800, 2500, 3200, and 3800 sequence fragments, and the number of unique assembled base pairs was determined. Based on the equations described above, an ideal plot of the number of base pairs remaining to be sequenced as a function of the # of sequenced fragments obtained with an average read length of 460 bp for a  $2.5 \times 10^6$  and a  $1.9 \times 10^6$  bp genome was determined (Figure 3). The progression of assembly was plotted using the actual data obtained from the assembly of up to 3800 sequence fragments and compared the data that is provided in the ideal plot (Figure 3). Figure 3 illustrates that there was essentially no deviation of the actual assembly data from the ideal plot, indicating that we had constructed close to an ideal random library with minimal contamination from double insert chimeras and free of vector.

### 3. Random DNA Sequencing

High quality double stranded DNA plasmid templates (19,687) were prepared using a "boiling bead" method developed in collaboration with Advanced Genetic Technology Corp. (Gaithersburg, MD) (Adams *et al.*, *Science* 252:1651 (1991); Adams *et al.*, *Nature* 355:632 (1992)). Plasmid preparation was performed in a 96-well format for all stages of DNA preparation from bacterial growth through final DNA purification. Template concentration was determined using Hoechst Dye and a Millipore Cytofluor. DNA concentrations were not adjusted, but low-yielding templates were identified where possible and not sequenced. Templates were also prepared from two *H. influenzae* lambda genomic libraries. An amplified library was constructed in vector Lambda GEM-12 (Promega) and an unamplified library was constructed in Lambda DASH II (Stratagene). In particular, for the



unamplified lambda library, *H. influenzae* Rd KW20 DNA (> 100 kb) was partially digested in a reaction mixture (200  $\mu$ l) containing 50  $\mu$ g DNA, 1X *Sau*3AI buffer, 20 units *Sau*3AI for 6 min. at 23°. The digested DNA was phenol-extracted and electrophoresed on a 0.5% low melting agarose gel at 2V/cm for 7 hours. Fragments from 15 to 25 kb were excised and recovered in a final volume of 6  $\mu$ l. One  $\mu$ l of fragments was used with 1  $\mu$ l of DASHII vector (Stratagene) in the recommended ligation reaction. One  $\mu$ l of the ligation mixture was used per packaging reaction following the recommended protocol with the Gigapack II XL Packaging Extract (Stratagene, #227711). Phage were plated directly without amplification from the packaging mixture (after dilution with 500  $\mu$ l of recommended SM buffer and chloroform treatment). Yield was about  $2.5 \times 10^3$  pfu/ $\mu$ l. The amplified library was prepared essentially as above except the lambda GEM-12 vector was used. After packaging, about  $3.5 \times 10^4$  pfu were plated on the restrictive NM539 host. The lysate was harvested in 2 ml of SM buffer and stored frozen in 7% dimethylsulfoxide. The phage titer was approximately  $1 \times 10^9$  pfu/ml.

Liquid lysates (10 ml) were prepared from randomly selected plaques and template was prepared on an anion-exchange resin (Qiagen). Sequencing reactions were carried out on plasmid templates using the AB Catalyst LabStation with Applied Biosystems PRISM Ready Reaction Dye Primer Cycle Sequencing Kits for the M13 forward (M13-21) and the M13 reverse (M13RP1) primers (Adams *et al.*, *Nature* 368:474 (1994)). Dye terminator sequencing reactions were carried out on the lambda templates on a Perkin-Elmer 9600 Thermocycler using the Applied Biosystems Ready Reaction Dye Terminator Cycle Sequencing kits. T7 and SP6 primers were used to sequence the ends of the inserts from the Lambda GEM-12 library and T7 and T3 primers were used to sequence the ends of the inserts from the Lambda DASH II library. Sequencing reactions (28,643) were performed by eight individuals using an average of fourteen AB 373 DNA Sequencers per day over a 3 month period. All sequencing reactions were analyzed using the Stretch modification of the AB 373, primarily using a 34 cm well-to-read distance. The overall

sequencing success rate was 84% for M13-21 sequences, 83% for M13RP1 sequences and 65% for dye-terminator reactions. The average usable read length was 485 bp for M13-21 sequences, 444 bp for M13RP1 sequences, and 375 bp for dye-terminator reactions. Table 5 summarizes the high-throughput sequencing phase of the invention.

Richards *et al.* (Richards *et al.*, *Automated DNA sequencing and Analysis*, M.D. Adams, C. Fields, J.C. Venter, Eds. (Academic Press, London, 1994), Chap. 28.) described the value of using sequence from both ends of sequencing templates to facilitate ordering of contigs in shotgun assembly projects of lambda and cosmid clones. We balanced the desirability of both-end sequencing (including the reduced cost of lower total number of templates) against shorter read-lengths for sequencing reactions performed with the M13RP1 (reverse) primer compared to the M13-21 (forward) primer. Approximately one-half of the templates were sequenced from both ends. In total, 9,297 M13RP1 sequencing reactions were done. Random reverse sequencing reactions were done based on successful forward sequencing reactions. Some M13RP1 sequences were obtained in a semi-directed fashion: M13-21 sequences pointing outward at the ends of contigs were chosen for M13RP1 sequencing in an effort to specifically order contigs. The semi-directed strategy was effective, and clone-based ordering formed an integral part of assembly and gap closure (see below).

#### 4. Protocol for Automated Cycle Sequencing

The sequencing consisted of using eight ABI Catalyst robots and fourteen AB 373 Automated DNA Sequencers. The Catalyst robot is a publicly available sophisticated pipetting and temperature control robot which has been developed specifically for DNA sequencing reactions. The Catalyst combines pre-aliquoted templates and reaction mixes consisting of deoxy- and dideoxynucleotides, the Taq thermostable DNA polymerase, fluorescently-labelled sequencing primers, and reaction buffer. Reaction mixes and

5 templates were combined in the wells of an aluminum 96-well thermocycling plate. Thirty consecutive cycles of linear amplification (e.g., one primer synthesis) steps were performed including denaturation, annealing of primer and template, and extension of DNA synthesis. A heated lid with rubber gaskets on the thermocycling plate prevented evaporation without the need for an oil overlay.

10 Two sequencing protocols were used: dye-labelled primers and dye-labelled dideoxy chain terminators. The shotgun sequencing involves use of four dye-labelled sequencing primers, one for each of the four terminator nucleotide. Each dye-primer is labelled with a different fluorescent dye, permitting the four individual reactions to be combined into one lane of the 373 DNA Sequencer for electrophoresis, detection, and base-calling. AB currently supplies pre-mixed reaction mixes in bulk packages containing all the necessary non-template reagents for sequencing. Sequencing can be done with 15 both plasmid and PCR-generated templates with both dye-primers and dye-terminators with approximately equal fidelity, although plasmid templates generally give longer usable sequences.

20 Thirty-two reactions were loaded per 373 Sequencer each day, for a total of 960 samples. Electrophoresis was run overnight following the manufacture's protocols, and the data was collected for twelve hours. Following electrophoresis and fluorescence detection, the AB 373 performs automatic lane tracking and base-calling. The lane-tracking was confirmed visually. Each sequence electropherogram (or fluorescence lane trace) was inspected visually and assessed for quality. Trailing sequences of low quality 25 were removed and the sequence itself was loaded via software to a Sybase database (archived daily to a 8mm tape). Leading vector polylinker sequence was removed automatically by software program. Average edited lengths of sequences from the standard ABI 373 were around 400 bp and depended mostly on the quality of the template used for the sequencing reaction. All of 30 the ABI 373 Sequencers were converted to Stretch Liners, which provided a

longer electrophoresis path prior to fluorescence detection, thus increasing the average number of usable bases to 500-600 bp.

## ***Informatics***

### ***1. Data Management***

5           A number of information management systems (LIMA) for a large-scale sequencing lab have been developed (Kerlavage *et al.*, *Proceedings of the Twenty-Sixth Annual Hawaii International Conference on System Sciences*, IEEE Computer Society Press, Washington D.C., 585 (1993)). The system used to collect and assemble the sequence data was developed using the Sybase  
10           relational data management system and was designed to automate data flow wherever possible and to reduce user error. The database stores and correlates all information collected during the entire operation from template preparation to final analysis of the genome. Because the raw output of the AB  
15           373 Sequencers was based on a Macintosh platform and the data management system chosen was based on a Unix platform, it was necessary to design and implement a variety of multi-user, client server applications which allow the raw data as well as analysis results to flow seamlessly into the database with a minimum of user effort. A description of the software programs used for large sequence assembly and management is provided in Figure 4.

### ***2. Assembly***

20           An assembly engine (**TIGR Assembler**) was developed for the rapid and accurate assembly of thousands of sequence fragments. The AB **AutoAssembler™** was modified (and named **TIGR Editor**) to provide a graphical interface to the electropherogram for the purpose of editing data  
25           associated with the aligned sequence file output of **TIGR Assembler**. **TIGR Editor** maintains synchrony between the electropherogram files on the

Macintosh platform and the sequence data in the *H. influenzae* database on the Unix platform.

5       The **TIGR assembler** simultaneously clusters and assembles fragments of the genome. In order to obtain the speed necessary to assemble more than 10<sup>4</sup> fragments, the algorithm builds a hash table of 10 bp oligonucleotide subsequences to generate a list of potential sequence fragment overlaps. The number of potential overlaps for each fragment determines which fragments are likely to fall into repetitive elements. Beginning with a single seed sequence fragment, **TIGR Assembler** extends the current contig by attempting  
10       to add the best matching fragment based on oligonucleotide content. The current contig and candidate fragment are aligned using a modified version of the Smith-Waterman algorithm (Waterman, M.S., *Methods in Enzymology* 164:765 (1988)) which provides for optimal gapped alignments. The current contig is extended by the fragment only if strict criteria for the quality of the  
15       match are met. The match criteria include the minimum length of overlap, the maximum length of an unmatched end, and the minimum percentage match. These criteria are automatically lowered by the algorithm in regions of minimal coverage and raised in regions with a possible repetitive element. The number of potential overlaps for each fragment determines which fragments  
20       are likely to fall into repetitive elements. Fragments representing the boundaries of repetitive elements and potentially chimeric fragments are often rejected based on partial mismatches at the ends of alignments and excluded from the current contig. **TIGR Assembler** is designed to take advantage of clone size information coupled with sequencing from both ends of each  
25       template. It enforces the constraint that sequence fragments from two ends of the same template point toward one another in the contig and are located within a certain ranged of base pairs (definable for each clone based on the known clone size range for a given library). Assembly of 24,304 sequence fragments of *H. influenzae* required 30 hours of CPU time using one processor  
30       on a SPARCenter 2000 with 512 Mb of RAM. This process resulted in approximately 210 contigs. Because of the high stringency of the **TIGR**

Assembler, all contigs were searched against each other using *grasta* (a modified *fasta* (Person and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444 (1988)). In this way, additional overlaps were detected which enabled compression of the data set into 140 contigs. The location of each fragment in the contigs and extensive information about the consensus sequence itself were loaded into the *H. influenzae* relational database.

### 3. *Ordering Assembled Contigs*

After assembly the relative positions of the 140 contigs were unknown. The contigs were ordered by *asmalign*. *Asmalign* uses a number of relationships to identify and align contigs that are adjacent to each other. Using this algorithm, the 140 contigs were placed into 42 groups totaling 42 physical gaps (no template DNA for the region) and 98 sequence gaps (template available for gap closure).

#### *Ordering Contigs Separated by Physical Gaps and Achieving Closure*

Four integrated strategies were developed to order contigs separated by physical gaps. Oligonucleotide primers were designed and synthesized from the end of each contig group. These primers were then available for use in one or more of the strategies outlined below:

1. Southern analysis was done to develop a unique "fingerprint" for a subset of 72 of the above oligonucleotides. This procedure was based upon the supposition that labeled oligonucleotides homologous to the ends of adjacent contigs should hybridize to common DNA restriction fragments, and thus share a similar or identical hybridization pattern or "fingerprint". Oligonucleotides were labeled using 50 pmoles of each 20 mer and 250 mCi of [ $\gamma$ - $^{32}$ P]ATP and T4 polynucleotide kinase. The labeled oligonucleotides were purified using Sephadex G-25 superfine (Pharmacia) and 107 cpm of each was used in a Southern hybridization analysis of *H. influenzae* Rd

chromosomal DNA digested with one frequent cutters (*AseI*) and five less frequent cutters (*BglIII*, *EcoRI*, *PstI*, *XbaI*, and *PvuII*). The DNA from each digest was fractionated on a 0.7% agarose gel and transferred to Nytran Plus nylon membranes (Schleicher & Schuell). Hybridization was carried out for 16 hours at 40°. To remove non-specific signals, each blot was sequentially washed at room temperature with increasingly stringent conditions up to 0.1X SSC + 0.5% SDS. Blots were exposed to a PhosphorImager cassette (Molecular Dynamics) for several hours and hybridization patterns were visually compared.

Adjacent contigs identified in this manner were targeted for specific PCR reactions.

2. Peptide links were made by searching each contig end using **blastx** (Altschul *et al.*, *J. Mol. Biol.* 215:403 (1990)) against a peptide database. If the ends of two contigs matched the same database sequence in an appropriate manner, then the two contigs were tentatively considered to be adjacent to each other.

3. The two lambda libraries constructed from *H. influenzae* genomic DNA were probed with oligonucleotides designed from the ends of contig groups (Kirkness *et al.*, *Genomics* 10:985 (1991)). The positive plaques were then used to prepare templates and the sequence was determined from each end of the lambda clone insert. These sequence fragments were searched using **grasta** against a database of all contigs. Two contigs that matched the sequence from the opposite ends of the same lambda clone were ordered. The lambda clone then provided the template for closure of the sequence gap between the adjacent contigs. The lambda clones were especially valuable for solving repeat structures.

4. To confirm the order of contigs found by the other approaches and establish the order of non-ordered contigs, standard and long range (XL) PCR reactions were performed as follows.

Standard PCR was performed in the following manner. Each reaction contained a 37 µl cocktail; 16.5 µl H<sub>2</sub>O, 3 µl 25 mM MgCl<sub>2</sub>, 8 µl of a dNTP

5 mix (1.25 mM each dNTP), 4.5  $\mu$ l 10X PCR core buffer II (Perkin Elmer), 25 ng *H. influenzae* Rd KW20 genomic DNA. The appropriate two primers (4  $\mu$ l, 3.2 pmole/ $\mu$ l) were added to each reaction. A hot start was performed at 95° for 5 min followed by a 75° hold. During the hold Amplitaq DNA polymerase (Perkin Elmer) 0.3  $\mu$ l in 4.3  $\mu$ l H<sub>2</sub>O, 0.5  $\mu$ l 10X PCR core buffer II, was added to each reaction. The PCR profile was 25 cycles of 94°/45 sec., denature; 55°/1 min., anneal; 72°/3 min, extension. All reactions were performed in a 96 well format on a Perkin Elmer GeneAmp PCR System 9600.

10 Long range PCR (XL PCR) was performed as follows: Each reaction contained a 35.2  $\mu$ l cocktail; 12.0  $\mu$ l H<sub>2</sub>O, 2.2  $\mu$ l 25 mM Mg(OAc)<sub>2</sub>, 4  $\mu$ l of a dNTP mix (200  $\mu$ M final concentration), 12.0  $\mu$ l 3.3X PCR buffer, 25 ng *H. influenzae* Rd KW20 genomic DNA. The appropriate two primers (5  $\mu$ l, 3.2 pmoles/ $\mu$ l) was added to each reaction. A hot start was performed at 94°  
15 for 1 minute. *rTth* polymerase, 2.0  $\mu$ l (4 U/reaction) in 2.8  $\mu$ l 3.3X PCR buffer II was added to each reaction. The PCR profile was 18 cycles of 94°/15 sec., denature; 62°/8 min., anneal and extend followed by 12 cycles 94°/15 sec., denature; 62°/8 min. (increase 15 sec./cycle), anneal and extend; 72°/10 min., final extension. All reactions were performed in a 96 well  
20 format on a Perkin Elmer GeneAmp PCR System 9600.

Although a PCR reaction was performed for essentially every combination of physical gap ends, techniques such as Southern fingerprinting, database matching, and the probing of large insert clones were particularly  
25 valuable in ordering contigs adjacent to each other and reducing the number of combinatorial PCR reactions necessary to achieve complete gap closure. Employing these strategies to an even greater extent in future genome projects will increase the overall efficiency of complete genome closure. The number of physical gaps ordered and closed by each of these techniques is summarized in Table 5.

30 Sequence information from the ends of 15-20 kb clones is particularly suitable for gap closure, solving repeat structures, and providing general



confirmation of the overall genome assembly. We were also concerned that some fragments of the *H. influenzae* genome would be non-clonable in a high copy plasmid in *E. coli*. We reasoned that lytic lambda clones would provide the DNA for these segments. Approximately 100 random plaques were picked from the amplified lambda library, templates prepared, and sequence information obtained from each end. These sequences were searched (grasta) against the contigs and linked in the database to their appropriate contig, thus providing a scaffolding of lambda clones contributing additional support to the accuracy of the genome assembly (Fig 5). In addition to confirmation of the contig structure, the lambda clones provided closure for 23 physical gaps. Approximately 78% of the genome is covered by lambda clones.

Lambda clones were also useful for solving repeat structures. Repeat structures identified in the genome were small enough to be spanned by a single clone from the random insert library, except for the six ribosomal RNA operons and one repeat (2 copies) which was 5,340 bp in length. Oligonucleotide probes were designed from the unique flanks at the beginning of each repeat and hybridized to the lambda libraries. Positive plaques were identified for each flank and the sequence fragments from the ends of each clone were used to correctly orient the repeats within the genome.

The ability to distinguish and assemble the six ribosomal RNA (rRNA) operons of *H. influenzae* (16S subunit-23S subunit-5S subunit) was a test of our overall strategy to sequence and assemble a complex genome which might contain a significant number of repeat regions. The high degree of sequence similarity and the length of the six operons caused the assembly process to cluster all the underlying sequences into a few indistinguishable contigs. To determine the correct placement of the operons in the sequence, a pair of unique flanking sequences was required for each. No unique flanking sequences could be found at the left (16S rRNA) ends. This region contains the ribosomal promoter and appeared to be non-clonable in the high copy number pUC18 plasmid. However, unique sequences could be identified at the right (5S) ends. Oligonucleotide primers were designed from these six

flanking regions and used to probe the two lambda libraries. For each of the six rRNA operons at least one positive plaque was identified which completely spanned the rRNA operon and contained unique flanking sequence at the 16S and 5S ends. These plaques provided the templates for obtaining the unique sequence for each of the six rRNA operons.

An additional confirmation of the global structure of the assembled circular genome was obtained by comparing a computer generated restriction map based on the assembled sequence for the enzymes *Apal*, *SmaI*, and *RsrII* with the predicted physical map of Redfield and Lee (*Genetic Maps: locus maps of complex genomes*, S.J. O'Brien, Ed. Cold Spring Harbor Laboratory Press, New York, N.Y., 1990, 2110.). The restriction fragments from the sequence-derived map matched those from the physical map in size and relative order (Figure 5).

### ***Editing***

Simultaneous with the final gap filling process, each contig was edited visually by reassembling overlapping 10 kb sections of contigs using the AB AutoAssembler™ and the Fast Data Finder™ hardware. AutoAssembler™ provides a graphical interface to electropherogram data for editing. The electropherogram data was used to assign the most likely base at each position. Where a discrepancy could not be resolved or a clear assignment made, the automatic base calls were left unchanged. Individual sequence changes were written to the electropherogram files and a replication protocol (**crash**) was used to maintain the synchrony of sequence data between the *H. influenzae* database and the electropherogram files. Following editing, contigs were reassembled with TIGR Assembler prior to annotation.

Potential frameshifts identified in the course of annotating the genome were saved as reports in the database. These reports include the coordinates in a contig which the alignment software (**praze**) predicts to be the most likely location of a missing or inserted base and a representation of the sequence

alignment containing the frameshift. Apparent frameshifts were used to indicate areas of the sequence which may require further editing. Frameshifts were not corrected in cases where clear electropherogram data disagreed with a frameshift. Frameshift editing was performed with **TIGR Editor**.

5           The rRNA and other repeat regions precluded complete assembly of the circular genome with **TIGR Assembler**. Final assembly of the genome was accomplished using **comb\_asm** which splices together contigs based on short overlaps.

### *Accuracy of the Genome Sequence*

10           The accuracy of the *H. influenzae* genome sequence is difficult to quantitate because there is very little previously determined *H. influenzae* sequence and most of these sequences are from other strains. There are, however, three parameters of accuracy that can be applied to the data. First, the number of apparent frameshifts in predicted *H. influenzae* genes, based on  
15           database similarities, is 148. Some of these apparent frameshifts may be in the database sequences rather than in ours, particularly considering that 49 of the apparent frameshifts are based on matches to hypothetical proteins from other organisms. Second, there are 188 bases in the genome that remain as N ambiguities (1/9,735 bp). Combining these two types of "known" errors, we  
20           can calculate a maximum sequence accuracy of 99.98%. The average coverage is 6.5X and less than 1% of the genome is single-fold coverage.

### *Identifying Genes*

25           An attempt was made to predict all of the coding regions of the *H. influenzae* Rd genome and identify genes, tRNAs and rRNAs, as well as other features of the DNA sequence (e.g., repeats, regulatory sites, replication origin sites, nucleotide composition). A description of some of the readily apparent sequence features is provided below.

The *H. influenzae* Rd genome is a circular chromosome of 1,830,121 bp. The overall G/C nucleotide content is approximately 38% (A = 31%, C = 19%, G = 19%, T = 31%, IUB = 0.035%). The G/C content of the genome was examined with several window lengths to look for global structural features. With a window of 5,000 bp, the G/C content is relatively even except for 7 large G/C-rich regions and several A/T-rich regions (Fig. 5). The G/C rich regions correspond to six rRNA operons and the location of a cryptic mu-like prophage. Genes for several proteins with similarity to proteins encoded by bacteriophage mu are located at approximately position 1.56-1.59 Mbp of the genome. This area of the genome has a markedly higher G/C content than average for *H. influenzae* (~50% G/C compared to ~38% for the rest of the genome). No significance has yet been ascertained for the source or importance of the A/T rich regions.

The minimal origin of replication (*oriC*) in *E. coli* is a 245 bp region defined by three copies of a thirteen base pair repeat containing a GATC core sequence at one end and four copies of a nine base pair repeat containing a TTAT core sequence at the other end. The GATC sites are methylation targets and control replication while the TTAT sites provide the binding sites for DnaA, the first step in the replication process (*Genes V*, B. Lewin Ed. (Oxford University Press, New York, 1994), chap. 18-19). An approximately 281 bp sequence (602,483 - 602,764) whose limits are defined by these same core sequences appears to define the origin of replication in *H. influenzae* Rd. These coordinates lie between sets of ribosomal operons *rrnF*, *rrnE*, *rrnD* and *rrnA*, *rrnB*, *rrnC*. These two groups of ribosomal operons are transcribed in opposite directions and the placement of the origin is consistent with their polarity for transcription. Termination of *E. coli* replication is marked by two 23 bp termination sequences located ~100 kb on either side of the midway point at which the two replication forks meet. Two potential termination sequences sharing a 10 bp core sequence with the *E. coli* termination sequence were identified in *H. influenzae* at coordinates 1,375,949-1,375,958 and 1,558,759-1,558,768. These two sets of coordinates are offset approximately

100 kb from the point 180° opposite of the proposed origin of *H. influenzae* replication.

Six rRNA operons were identified. Each rRNA operon contains three rRNA subunits and a variable spacer region in the order: 16S subunit - spacer region - 23S subunit - 5S subunit. The subunit lengths are 1539 bp, 2653 bp, and 116 bp, respectively. The G/C content of the three ribosomal subunits (50%) is higher than the genome as a whole. The G/C content of the spacer region (38%) is consistent with the remainder of the genome. The nucleotide sequence of the three rRNA subunits is 100% identical in all six ribosomal operons. The rRNA operons can be grouped into two classes based on the spacer region between the 16S and 23S sequences. The shorter of the two spacer regions is 478 bp in length (*rrnB*, *rrnE*, and *rrnF*) and contains the gene for tRNA Glu. The longer spacer is 723 bp in length (*rrnA*, *rrnC*, and *rrnD*) and contains the genes for tRNA Ile and tRNA Ala. The two sets of spacer regions are also 100% identical across each group of three operons. tRNA genes are also present at the 16S and 5S ends of two of the rRNA operons. The genes for tRNA Arg, tRNA His, and tRNA Pro are located at the 16S end of *rrnE* while the genes for tRNA Trp, and tRNA Asp are located at the 5S end of *rrnA*.

The predicted coding regions of the *H. influenzae* genome were initially defined by evaluating their coding potential with the program Genemark (Borodovsky and McIninch, *Computers Chem.* 17(2):123 (1993)) using codon frequency matrices derived from 122 *H. influenzae* coding sequences in GenBank. The predicted coding region sequences (plus 300 bp of flanking sequence) were used in searches against a database of non-redundant bacterial proteins (NRBP) created specifically for the annotation. Redundancy was removed from NRBP at two stages. All DNA coding sequences were extracted from GenBank (release 85), and sequences from the same species were searched against each other. Sequences having >97% similarity over regions >100 nucleotides were combined. In addition, the sequences were translated and used in protein comparisons with all sequences in Swiss-Prot

(release 30). Sequences belonging to the same species and having >98% similarity over 33 amino acids were combined. NRBP is composed of 21,445 sequences extracted from 23,751 GenBank sequences and 11,183 Swiss-Prot sequences from 1,099 different species.

5 A total of 1,749 predicted coding regions were identified. Searches of the *H. influenzae* predicted coding regions were performed using an algorithm that translates the query DNA sequence in the three plus-strand reading frames for searching against NRBP, identifies the protein sequences that match the query, and aligns the protein-protein matches using **praze**, a modified Smith-  
10 Waterman (Pearson and Lipman, *Proc. Natl. Acad. Sci. U.S.A.* 85:2444 (1988)) algorithm. In cases where insertion or deletions in the DNA sequence produced a frameshift error, the alignment algorithm started with protein regions of maximum similarity and extended the alignment to the same database match in alternative frames using the 300 bp flanking region.  
15 Regions known to contain frameshift errors were saved in the database and evaluated for possible correction. Unidentified predicted coding regions and the remaining intergenic sequences were searched against a dataset of all available peptide sequences from Swiss-Prot, PIR, and GenBank. Identification of operon structures will be facilitated by experimental  
20 determination of transcription promoter and termination sites.

Each putatively identified *H. influenzae* gene was assigned to one of 102 biological role categories adapted from Riley (Riley, M., *Microbiology Reviews* 57(4):862 (1993)). Assignments were made by linking the protein sequence of the predicted coding regions with the Swiss-Prot sequences in the  
25 Riley database. Of the 1,749 predicted coding regions, 724 have no role assignment. Of these, no database match was found for 384, while 340 matched "hypothetical proteins" in the database. Role assignments were made for 1,025 of the predicted coding regions. A compilation of all the predicted coding regions, their unique identifiers, a three letter gene identifier, percent  
30 identity, percent similarity, and amino acid match length are presented in Table 1(a).

An annotated complete genome map of *H. influenzae* Rd is presented in Figures 6(A)-(D). The map places each predicted coding region on the *H. influenzae* chromosome, indicates its direction of transcription and color codes its role assignment. Role assignments are also represented in Figure 5.

5 A survey of the genes and their chromosomal organization in *H. influenzae* Rd make possible a description of the metabolic processes *H. influenzae* requires for survival as a free living organism, the nutritional requirements for its growth in the laboratory, and the characteristics which make it unique from other organisms specifically as it relates to its  
10 pathogenicity and virulence. The genome would be expected to have complete complements of certain classes of genes known to be essential for life. For example, there is a one-to-one correspondence of published *E. coli* ribosomal protein sequences to potential homologs in the *H. influenzae* database. Likewise, as shown in Table 1(a), an aminoacyl tRNA-synthetase is present  
15 in the genome for each amino acid. Finally, the location of tRNA genes was mapped onto the genome. There are 54 identified tRNA genes, including representatives of all 20 amino acids.

In order to survive as a free living organism, *H. influenzae* must produce energy in the form of ATP via fermentation and/or electron transport.  
20 As a facultative anaerobe, *H. influenzae* Rd is known to ferment glucose, fructose, galactose, ribose, xylose and fucose (Dorocicz *et al.*, *J. Bacteriol.* 175:7142 (1993)). The genes identified in Table 1(a) indicate that transport systems are available for the uptake of these sugars via the phosphoenolpyruvate-phosphotransferase system (PTS), and via non-PTS  
25 mechanisms. Genes that specify the common phosphate-carriers Enzyme I and Hpr (*ptsI* and *ptsH*) of the PTS system were identified as well as the glucose specific *crr* gene. The *ptsH*, *ptsI*, and *crr* genes constitute the *pts* operon. We have not however identified the gene encoding membrane-bound glucose specific Enzyme II. The latter enzyme is required for transport of glucose by  
30 the PTS system. A complete PTS system for fructose was identified.

Genes encoding the complete glycolytic pathway and for the production of fermentative end products were identified. Growth utilizing anaerobic respiratory mechanisms were found by identifying genes encoding functional electron transport systems using inorganic electron acceptors such as nitrates, nitrites, and dimethylsulfoxide. Genes encoding three enzymes of the tricarboxylic acid (TCA) cycle appear to be absent from the genome. Citrate synthase, isocitrate dehydrogenase, and acortase were not found by searching the predicted coding regions or by using the *E. coli* enzymes as peptide queries against the entire genome in translation. This provides an explanation for the very high level of glutamate (lg/L) which is required in defined culture media (Klein and Luginbuhl, *J. Gen. Microbiol.* 113:409 (1979)). Glutamate can be directed into the TCA cycle via conversion to alpha-ketoglutarate by glutamate dehydrogenase. In the absence of a complete TCA cycle, glutamate presumably serves as the source of carbon for biosynthesis of amino acids using precursors which branch from the TCA cycle. Functional electron transport systems are available for the production of ATP using oxygen as a terminal electron acceptor.

Previously unanswered questions regarding pathogenicity and virulence can be addressed by examining certain classes of genes such as adhesions and the lipooligosaccharide biogenesis genes. Moxon and co-workers (Weiser *et al.*, *Cell* 59:657 (1989)) have obtained evidence that a number of these virulence-related genes contain tandem tetramer repeats which undergo frequent addition and deletion of one or more repeat units during replication such that the reading frame of the gene is changed and its expression thereby altered. It is now possible, using the complete genome sequence, to locate all such tandem repeat tracts (Figure 5) and to begin to determine their roles in phase variation of such potential virulence genes.

*H. influenzae* Rd possesses a highly efficient natural DNA transformation system (Kahn and Smith, *J. Membrane Biol.* 138:155 (1984)). A unique DNA uptake sequence site, 5' AAGTGCGGT, present in multiple copies in the genome, has been shown to be necessary for efficient DNA



uptake. It is now possible to locate all of these sites and completely describe their distribution with respect to genic and intergenic regions. Fifteen genes involved in transformation have already been described and sequenced (Redfield, R., *J. Bacteriol.* 173:5612 (1991); Chandler, M., *Proc. Natl. Acad. Sci. U.S.A* 89:1616 (1992); Barouki and Smith, *J. Bacteriol.* 163(2):629 (1985); Tomb *et al.*, *Gene* 104:1 (1991); Tomb, J, *Proc. Natl. Acad. Sci. U.S.A* 89:10252 (1992)). Six of the genes, *comA* to *comF*, comprise an operon which is under positive control by a 22-bp palindromic competence regulatory element (CRE) about one helix turn upstream of the promoter. The *rec-2* transformation gene is also controlled by this element. It is now possible to locate additional copies of CRE in the genome and discover potential transformation genes under CRE control. In addition, it may now be possible to discover other global regulatory elements with an ease not previously possible.

One well-described gene regulatory system in bacteria is the "two-component" system composed of a sensor molecule that detects some sort of environmental signal and a regulator molecule that is phosphorylated by the activated form of the sensor. The regulator protein is generally a transcription factor which, when activated by the sensor, turns on or off expression of a specific set of genes (for review, see Albright *et al.*, *Ann. Rev. Genet.* 23:311 (1989); Parkinson and Kofoed, *Ann. Rev. Genet.* 26:71 (1992)). It has been estimated that *E. coli* harbors 40 sensor-regulator pairs (Albright *et al.*, *Ann. Rev. Genet.* 23:311 (1989); Parkinson and Kofoed, *Ann. Rev. Genet.* 26:71 (1992)). The *H. influenzae* genome was searched with representative proteins from each family of sensor and regulator proteins using *tblastn* and *tfasta*. Four sensor and five regulator proteins were identified with similarity to proteins from other species (Table 6). There appears to be a corresponding sensor for each regulator protein except CpxR. Searches with the CpxA protein from *E. coli* identified three of the four sensors listed in Table 6, but no additional significant matches were found. It is possible that the level of sequence similarity is low enough to be undetectable with *tfasta*. No

representatives of the NtrC-class of regulators were found. This class of proteins interacts directly with the sigma-54 subunit of RNA polymerase, which is not present in *H. influenzae*. All of the regulator proteins fall into the OmpR subclass (Albright *et al.*, *Ann. Rev. Genet.* 23:311 (1989); Parkinson and Kofoed, *Ann. Rev. Genet.* 26:71 (1992)). The *phoBR* and *basRS* genes of *H. influenzae* are adjacent to one another and presumably form an operon. The *nar* and *arc* genes are not located adjacent to one another.

Some of the most interesting questions that can be answered by a complete genome sequence relate to what genes or pathways are absent. The non-pathogenic *H. influenzae* Rd strain varies significantly from the pathogenic serotype b strains. Many of the differences between these two strains appear in factors affecting infectivity. For example, the eight genes which make up the fimbrial gene cluster (vanHam *et al.*, *Mol. Microbiol.* 13:673 (1994)) involved in adhesion of bacteria to host cells are now shown to be absent in the Rd strain. The *pepN* and *purE* genes which flank the fimbrial cluster in *H. influenzae* type b strains are adjacent to one another in the Rd strain (Fig. 7), suggesting that the entire fimbrial duster was excised. On a broader level, we determined which *E. coli* proteins are not in *H. influenzae* by taking advantage of a non-redundant set of protein coding genes from *E. coli*, namely the University of Wisconsin Genome Project contigs in GenBank: 1,216 predicted protein sequences from GenBank accessions D10483, L10328, U00006, U00039, U14003, and U18997 (Yura *et al.*, *Nucleic Acids Research* 20:3305 (1992); Burland *et al.*, *Genomics* 16:551 (1993)). The minimum threshold for matches was set so that even weak matches would be scored as positive, thereby giving a minimal estimate of the *E. coli* genes not present in *H. influenzae*. *tblastn* was used to search each of the *E. coli* proteins against the complete genome. All *blast* scores >100 were considered matches. Altogether 627 *E. coli* proteins matched at least one region of the *H. influenzae* genome and 589 proteins did not. The 589 non-matching proteins were examined and found to contain a disproportionate number of hypothetical proteins from *E. coli*. Sixty-eight percent of the identified *E. coli* proteins

were matched by an *H. influenzae* sequence whereas only 38% of the hypothetical proteins were matched. Proteins are annotated as hypothetical based on a lack of matches with any other known protein (Yura *et al.*, *Nucleic Acids Research* 20:3305 (1992); Burland *et al.*, *Genomics* 16:551 (1993)). At least two potential explanations can be offered for the over representation of hypothetical proteins among those without matches: some of the hypothetical proteins are not, in fact, translated (at least in the annotated frame), or these are *E. coli*-specific proteins that are unlikely to be found in any species except those most closely related to *E. coli*, for example *Salmonella typhimurium*.

A total of 384 predicted coding regions did not display significant similarity with a six-frame translation of GenBank release 87. These unidentified coding regions were compared to one another with *fasta*. Several novel gene families were identified. For example, two predicted coding regions without database matches (HI0591, HI0852) share 75% identity over almost their entire lengths (139 and 143 amino acid residues respectively). Their similarity to each other but failure to match any protein available in the current databases suggest that they could represent a novel cellular function.

Other types of analyses can be applied to the unidentified coding regions, including hydropathy analysis, which indicates the patterns of potential membrane-spanning domains that are often conserved between members of receptor and transporter gene families, even in the absence of significant amino acid identity. Five examples of unidentified predicted coding regions that display potential transmembrane domains with a periodic pattern that is characteristic of membrane-bound channel proteins are shown in Figure 8. Such information can be used to focus on specific aspects of cellular function that are affected by targeted deletion or mutation of these genes.

Interest in the medically important aspects of *H. influenzae* biology has focused particularly on those genes which determine virulence characteristics of the organism. Recently, the catalase gene was characterized and sequenced as a possible virulence-related gene (Bishai *et al.*, *J. Bacteriol.* 176:2914

(1994)). A number of the genes responsible for the capsular polysaccharide have been mapped and sequenced (Kroll *et al.*, *Mol. Microbiol.* 5(6):1549 (1991)). Several outer membrane protein genes have been identified and sequenced (Langford *et al.*, *J. Gen. Microbiol.* 138:155 (1992)). The  
5 lipooligosaccharide component of the outer membrane and the genes of its synthetic pathway are under intensive study (Weiser *et al.*, *J. Bacteriol.* 173:3304 (1990)). While a vaccine is available, the study of outer membrane components is motivated to some extent by the need for improved vaccines.

#### ***Data Availability***

10 The *H. influenzae* genome sequence has been deposited in the Genome Sequence DataBase (GSDB) with the accession number L42023. The nucleotide sequence and peptide translation of each predicted coding region with identified start and stop codons have also been accessioned by GSDB.

#### ***Production of an Antibody to a Haemophilus influenzae Protein***

15 Substantially pure protein or polypeptide is isolated from the transfected or transformed cells using any one of the methods known in the art. The protein can also be produced in a recombinant prokaryotic expression system, such as *E. coli*, or can be chemically synthesized. Concentration of protein in the final preparation is adjusted, for example, by concentration on  
20 an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

#### ***Monoclonal Antibody Production by Hybridoma Fusion***

Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the  
25 classical method of Kohler, G. and Milstein, C., *Nature* 256:495 (1975) or

modifications of the methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, E., *Meth. Enzymol.* 70:419 (1980), and modified methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. *et al.* *Basic Methods in Molecular Biology* Elsevier, New York. Section 21-2 (1989).

#### ***Polyclonal Antibody Production by Immunization***

Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than other and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. *et al.*, *J. Clin. Endocrinol. Metab.* 33:988-991 (1971).

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. *et al.*, Chap. 5 19 in: *Handbook of Experimental Immunology*, Wier, D., ed, Blackwell (1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2 mg/ml of serum (about 12  $\mu$ M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: *Manual of Clinical Immunology*, second 10 edition, Rose and Friedman, eds., Amer. Soc. For Microbiology, Washington, D.C. (1980).

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively 15 or qualitatively to identify the presence of antigen in a biological sample.

#### ***Preparation of PCR Primers and Amplification of DNA***

Various fragments of the *Haemophilus influenzae* Rd genome, such as those disclosed in Tables 1(a) and 2 can be used, in accordance with the present invention, to prepare PCR primers for a variety of uses. The PCR 20 primers are preferably at least 15 bases, and more preferably at least 18 bases in length. When selecting a primer sequence, it is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approximately the same. The PCR primers and amplified DNA of this Example find use in the Examples that follow.

#### ***Gene expression from DNA Sequences Corresponding to ORFs***

A fragment of the *Haemophilus influenzae* Rd genome provided in Tables 1(a) or 2 is introduced into an expression vector using conventional

technology. (Techniques to transfer cloned sequences into expression vectors that direct protein translation in mammalian, yeast, insect or bacterial expression systems are well known in the art.) Commercially available vectors and expression systems are available from a variety of suppliers including  
5 Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence may be optimized for the particular expression organism, as explained by Hatfield *et al.*, U.S. Patent No. 5,082,767, incorporated herein  
10 by this reference.

The following is provided as one exemplary method to generate polypeptide(s) from cloned ORFs of the *Haemophilus* genome fragment. Since the ORF lacks a poly A sequence because of the bacterial origin of the ORF, this sequence can be added to the construct by, for example, splicing out  
15 the poly A sequence from pSG5 (Stratagene) using *Bgl*I and *Sal*I restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene) for use in eukaryotic expression systems. pXT1 contains the LTRs and a portion of the gag gene from Moloney Murine Leukemia Virus. The position of the LTRs in the construct allow efficient  
20 stable transfection. The vector includes the Herpes Simplex thymidine kinase promoter and the selectable neomycin gene. The *Haemophilus* DNA is obtained by PCR from the bacterial vector using oligonucleotide primers complementary to the *Haemophilus* DNA and containing restriction endonuclease sequences for *Pst*I incorporated into the 5' primer and *Bgl*II at  
25 the 5' end of the corresponding *Haemophilus* DNA 3' primer, taking care to ensure that the *Haemophilus* DNA is positioned such that its followed with the poly A sequence. The purified fragment obtained from the resulting PCR reaction is digested with *Pst*I, blunt ended with an exonuclease, digested with *Bgl*II, purified and ligated to pXT1, now containing a poly A sequence and  
30 digested *Bgl*II.

The ligated product is transfected into mouse NIH 3T3 cells using Lipofectin (Life Technologies, Inc., Grand Island, New York) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in 600 ug/ml G418 (Sigma, St. Louis, Missouri). The protein is preferably released into the supernatant. However if the protein has membrane binding domains, the protein may additionally be retained within the cell or expression may be restricted to the cell surface.

Since it may be necessary to purify and locate the transfected product, synthetic 15-mer peptides synthesized from the predicted *Haemophilus* DNA sequence are injected into mice to generate antibody to the polypeptide encoded by the *Haemophilus* DNA.

If antibody production is not possible, the *Haemophilus* DNA sequence is additionally incorporated into eukaryotic expression vectors and expressed as a chimeric with, for example,  $\beta$ -globin. Antibody to  $\beta$ -globin is used to purify the chimeric. Corresponding protease cleavage sites engineered between the  $\beta$ -globin gene and the *Haemophilus* DNA are then used to separate the two polypeptide fragments from one another after translation. One useful expression vector for generating  $\beta$ -globin chimerics is pSG5 (Stratagene). This vector encodes rabbit  $\beta$ -globin. Intron II of the rabbit  $\beta$ -globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression. These techniques as described are well known to those skilled in the art of molecular biology. Standard methods are published in methods texts such as Davis et al. and many of the methods are available from the technical assistance representatives from Stratagene, Life Technologies, Inc., or Promega. Polypeptide may additionally be produced from either construct using in vitro translation systems such as In vitro Express<sup>TM</sup> Translation Kit (Stratagene).

While the present invention has been described in some detail for purposes of clarity and understanding, one skilled in the art will appreciate that various



changes in form and detail can be made without departing from the true scope of the invention.

All patents, patent applications and publications referred to above are hereby incorporated by reference.

Amino acid biosynthesis						
	Glutamate family					
HI0190	202698	204044	glutamate dehydrogenase (gdhA) (Escherichia coli)	74.1	84.4	446
HI0867	915793	917833	glutamine synthetase (glnA) (Proteus vulgaris)	70.7	85.9	467
HI1725	1792409	1789821	uridylyl transferase (glnD) (Escherichia coli)	46.6	67.8	854
HI0813	861610	860240	argininosuccinate lyase (arginosuccinase) (asal) (argH) (Escherichia coli)	73.5	84.5	457
HI1733	1799112	1800443	argininosuccinate synthetase (argG) (Escherichia coli)	78.6	87.5	438
HI0598	618753	617752	omithine carbamoyltransferase (arcB) (Pseudomonas aeruginosa)	82.3	90.7	334
HI1242	1313013	1311763	gamma-glutamyl phosphate reductase (proA) (Escherichia coli)	61.7	79.4	406
HI0902	955518	956621	glutamate 5-kinase (gamma-glutamyl kinase) (proB) (Escherichia coli)	65.7	80.2	363

Table 1(a)

	Aspartate family						
HI0288	319209	320419	aspartate aminotransferase (aspC) (Bacillus sp.)		31.1	53.8	349
HI1623	1684147	1685334	aspartate aminotransferase (aspC) (Escherichia coli)		62.6	79.0	396
HI0566	582379	583368	asparagine synthetase A (asnA) (Escherichia coli)		63.3	77.0	330
HI0648	690744	689632	aspartate-semialdehyde dehydrogenase (asd) (Escherichia coli)		71.9	84.9	367
HI1311	1385700	1386509	dehydrodipicolinate reductase (dapB) (Escherichia coli)		70.3	82.5	269
HI0729	779456	778212	diaminopimelate decarboxylase (dap decarboxylase) (lysA) (Pseudomonas aeruginosa)		57.6	78.8	413
HI0752	810250	811071	diaminopimelate epimerase (dapF) (Escherichia coli)		77.0	85.8	274
HI0256	284972	285865	dihydrodipicolinate synthetase (dapA) (Escherichia coli)		58.2	79.8	292
HI1638	1693968	1694330	lysine-sensitive aspartokinase III (lysC) (Escherichia coli)		55.3	73.2	449
HI0102	109226	108096	succinyl-diaminopimelate desuccinylase (dapE) (Escherichia coli)		61.6	79.7	374
HI1640	1696728	1695820	tetrahydrodipicolinate N-succinyltransferase (dapD) (Actinobacillus pleuropneumoniae)		96.7	98.5	273
HI0089	96280	93836	aspartokinase-homoserine dehydrogenase (thrA) (Serratia marcescens)		62.2	77.4	814
HI0088	93820	92879	homoserine kinase (thrB) (Serratia marcescens)		61.8	80.6	306
HI0087	92833	91559	threonine synthase (thrC) (Serratia marcescens)		67.0	80.9	425
HI1044	1107725	1105876	B12-dependent homocysteine-N5-methyltetrahydrofolate transmethylation (meth) (Escherichia coli)		54.2	70.4	1217
HI0122	137932	136745	beta-cystathionase (metC) (Escherichia coli)		65.4	84.1	390
HI0086	90743	89601	cystathionine gamma-synthase (metB) (Escherichia coli)		41.9	62.2	374
HI1266	1339983	1341056	homoserine acetyltransferase (met2) (Saccharomyces cerevisiae)		38.1	57.1	387
HI1708	1773488	1771221	tetrahydropteroyltrimethylglutamate methyltransferase (metE) (Escherichia coli)		52.4	68.0	747

	Serine family					
HI0891	942366	943628	serine hydroxymethyltransferase (serine methylase) (glyA) (Actinobacillus actinomycetemcomitans)	85.7	93.6	419
HI0467	486594	487823	phosphoglycerate dehydrogenase (serA) (Escherichia coli)	71.1	83.9	408
HI1170	1238587	1237502	phosphoserine aminotransferase (serC) (Escherichia coli)	53.4	72.3	358
HI1035	1097573	1098514	phosphoserine phosphatase (o-phosphoserine phosphohydrolase) (serB) (Escherichia coli)	52.3	69.5	303
HI1105	1165130	1166077	cysteine synthetase (cysK) (Escherichia coli)	70.0	83.9	309
HI0608	636187	636987	serine acetyltransferase (cysE) (Escherichia coli)	73.0	88.3	256
	Aromatic amino acid family					
HI0972	1026936	1027382	3-dehydroquinase (aroQ) (Actinobacillus pleuropneumoniae)	67.1	82.5	143
HI0209	222169	223254	3-dehydroquininate synthase (aroB) (Escherichia coli)	62.1	76.7	356
HI0197	211424	212494	chorismate synthase (aroC) (Escherichia coli)	77.3	88.4	350
HI0609	637000	637812	dehydroquinase shikimate dehydrogenase (Nicotiana tabacum)	30.0	51.5	242
HI1595	1656463	1657758	enolpyruvylshikimatephosphatesynthase (aroA) (Haemophilus influenzae)	97.7	98.4	432
HI0657	698939	698124	shikimate 5-dehydrogenase (aroE) (Escherichia coli)	49.1	70.1	270
HI0208	221607	222146	shikimate acid kinase I (aroK) (Escherichia coli)	75.0	87.5	104

HI1148	1213767	1214921	chorismate mutase/prephenate dehydratase pheA polypeptide (pheA) (Escherichia coli)	54.3	74.7	375
HI1553	1618339	1617254	DAHPSynthetase (phenylalanine repressible) (aroG) (Escherichia coli)	72.0	83.8	345
HI1293	1370448	1371578	chorismate mutase (tyrA) (Erwinia herbicola)	58.6	76.8	366
HI1392	1481917	1483470	anthranilate synthase component I (trpE) (Escherichia coli)	52.9	73.2	494
HI1393	1483718	1485554	anthranilate synthase component II (trpD) (Escherichia coli)	56.6	74.2	452
HI1174	1240757	1241335	anthranilate synthase glutamine amidotransferase (trpG) (Acinetobacter calcoaceticus)	34.0	59.0	191
HI1437	1519794	1520597	tryptophan synthase alpha chain (trpA) (Salmonella typhimurium)	57.8	72.8	267
HI1436	1518601	1519791	tryptophan synthase, beta chain (trpB) (Escherichia coli)	82.4	90.3	391
HI0474	494758	495354	amidotransferase (hisH) (Escherichia coli)	55.9	70.3	195
HI0470	490033	490941	ATP phosphoribosyltransferase (hisG) (Escherichia coli)	72.2	82.0	295
HI0476	496124	496897	hisF cyclase (hisF) (Escherichia coli)	82.0	91.0	256
HI0472	492389	493489	histidinol-phosphate aminotransferase (hisC) (Escherichia coli)	60.1	77.5	351
HI1169	1237411	1236314	histidinol-phosphate aminotransferase (hisH) (Bacillus subtilis)	38.7	61.0	354
HI0473	493604	494689	imidazoleglycerol-phosphate dehydratase (hisB) (Escherichia coli)	65.0	80.5	353
HI0477	496900	497562	phosphoribosyl-AMP cyclohydrolase (hisE) (Escherichia coli)	60.7	77.0	195
HI0475	495393	496139	phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase (hisA) (Escherichia coli)	62.9	77.1	245

	Pyruvate family						
HI1581	1642613	1643692	alanine racemase, biosynthetic (alr) (Escherichia coli)		56.3	74.9	358
	Branched chain family						
HI0739	791174	791968	acetylhydroxy acid synthase II (ilvG) (Escherichia coli)		63.6	78.5	386
HI1591	1652923	1651205	acetylacetyl synthase III large chain (ilvI) (Escherichia coli)		69.1	83.9	527
HI1590	1651202	1650714	acetylacetyl synthase III small chain (ilvH) (Escherichia coli)		65.6	85.0	160
HI1196	1259031	1258003	branched-chain-amino-acid transaminase (Salmonella typhimurium)		32.9	49.8	298
HI0740	791969	793960	dihydroxyacid dehydratase (ilvD) (Escherichia coli)		77.9	89.5	614
HI0684	723320	724795	ketol-acid reductoisomerase (ilvC) (Escherichia coli)		81.7	89.6	491
HI0991	1047074	1047673	3-isopropylmalate dehydratase (isopropylmalate isomerase) (leuD) (Salmonella typhimurium)		71.1	86.3	197
HI0989	1044390	1045463	3-isopropylmalate dehydrogenase (beta-IPM dehydrogenase) (leuB) (Salmonella typhimurium)		68.0	80.1	353
HI0985	1040319	1039678	leuA protein (leuA) (Haemophilus influenzae)		99.5	100.0	193

Biosynthesis of cofactors, prosthetic groups, carriers									
	Biotin								
HI1560	1625092	1623803	7,8-diamino-pelargonic acid aminotransferase (bioA) (Escherichia coli)	58.0	74.1	420			
HI1559	1623791	1622652	7-keto-8-aminopelargonic acid synthetase (bioF) (Bacillus sphaericus)	33.5	56.3	370			
HI1557	1622004	1621225	biotin biosynthesis; reaction prior to pimeloyl CoA (bioC) (Escherichia coli)	28.6	46.8	151			
HI0645	687346	684872	biotin sulfoxide reductase (BDS reductase) (bisC) (Escherichia coli)	54.0	71.8	734			
HI1024	1085538	1086535	biotin synthetase (bioB) (Escherichia coli)	59.6	77.5	307			
HI1556	1621212	1620640	dehydrobiotin synthase (bioD) (Bacillus sphaericus)	42.1	59.6	175			
HI1449	1532932	1532207	dehydrobiotin synthetase (bioD) (Escherichia coli)	41.3	62.4	217			
	Folic acid								
HI1448	1531237	1532112	5,10-methylenetetrahydrofolate reductase (metF) (Escherichia coli)	72.8	83.4	290			
HI0611	640325	639480	5,10-methylene-tetrahydrofolate dehydrogenase (folD) (Escherichia coli)	67.6	82.0	278			
HI0064	67257	67760	7,8-dihydro-6-hydroxymethylpterin-pyrophosphokinase (folK) (Escherichia coli)	56.3	77.8	158			
HI0459	478432	477392	aminodeoxychorismate lyase (pabC) (Escherichia coli)	40.1	66.5	243			
HI1635	1691986	1691351	dedA protein (dedA) (Escherichia coli)	30.4	55.1	158			

HI0901	955417	954938	dehydrofolate reductase, type I (folA) (Escherichia coli)	53.2	68.4	158
HI1339	1412130	1412954	dihydropteroate synthase (folP) (Escherichia coli)	54.5	70.9	275
HI1469	1547395	1548370	dihydropteroate synthase (folP) (Escherichia coli)	54.5	70.9	275
HI1264	1337544	1338854	folypolyglutamate synthase (folC) (Escherichia coli)	51.7	68.4	409
HI1451	1534018	1533365	GTP cyclohydrolase I (folE) (Escherichia coli)	63.9	79.0	219
HI1173	1240715	1239732	p-aminobenzoate synthetase (pabB) (Escherichia coli)	31.0	53.6	257
	Lipoate					
HI0026	28610	27651	lipoate biosynthesis protein A (lipA) (Escherichia coli)	73.8	84.1	321
HI0027	29302	28667	lipoate biosynthesis protein B (lipB) (Escherichia coli)	66.7	84.2	181
	Molybdopterin					
HI1681	1743523	1743044	moaC protein (moaC) (Escherichia coli)	79.1	89.2	157
HI1682	1744628	1743618	molybdenum cofactor biosynthesis protein A (moaA) (Escherichia coli)	61.8	78.3	327
HI1373	1461582	1461376	molybdenum-pterin binding protein (mopI) (Clostridium pasteurianum)	51.5	74.2	66
HI1680	1743078	1742797	molybdopterin (MPT) converting factor, subunit 1 (moaD) (Escherichia coli)	59.3	79.0	81
HI1452	1534156	1535367	molybdopterin biosynthesis protein (chlE) (Escherichia coli)	56.4	72.5	403
HI0118	132351	133133	molybdopterin biosynthesis protein (chlN) (Escherichia coli)	27.9	52.9	135
HI1453	1535374	1536102	molybdopterin biosynthesis protein (chlN) (Escherichia coli)	63.9	78.4	241
HI1679	1742793	1742344	molybdopterin converting factor, subunit 2 (moaE) (Escherichia coli)	58.0	76.0	150
HI0846	892779	892204	molybdopterin-guanine dinucleotide (mob) (Escherichia coli)	39.4	61.7	187
	Pantothenate					
HI0633	670462	669530	anthothenate kinase (coaA) (Escherichia coli)	64.1	78.2	314



	Pyridoxine								
HI0865	913165	913851	pyridoxamine phosphate oxidase (pdxH) (Escherichia coli)		46.0	65.3	213		
	Riboflavin								
HI0766	827249	827893	3,4-dihydroxy-2-butanone 4-phosphate synthase (ribB) (Escherichia coli)		69.6	82.7	213		
HI0213	225991	226662	GTP cyclohydrolase II (ribA) (Escherichia coli)		68.0	81.4	193		
HI0946	1002768	1003883	riboflavin biosynthesis protein RIBG (ribD) (Escherichia coli)		57.6	76.5	361		
HI1619	1678899	1679510	riboflavin synthase alpha chain (ribC) (Escherichia coli)		65.5	82.3	203		
HI1306	1382553	1383071	riboflavin synthase beta chain (ribE) (Escherichia coli)		76.3	89.7	156		
	Thioredoxin, glutaredoxin, glutathione								
HI0162	177496	176129	glutathione reductase (gor) (Escherichia coli)		74.2	85.0	450		
HI1118	1181697	1181197	thioredoxin (trxA) (Anabaena sp.)		36.6	58.5	82		
HI1162	1228652	1228002	thioredoxin (trxA) (Anabaena sp.)		33.3	61.5	39		
HI0084	88470	88150	thioredoxin m (trxM) (Anacystis nidulans)		53.3	79.4	107		
	Menaquinone, ubiquinone								
HI0285	317765	316062	2-succinyl-6-hydroxy-2,4-cyclohexadiene-1-carboxylate synthase (menD) (Escherichia coli)		46.8	64.4	551		
HI0971	1025835	1026875	4-(2'-carboxyphenyl)-4-oxybutyric acid synthase (menC) (Escherichia coli)		57.3	74.2	312		
HI1192	1256548	1255916	coenzyme PQQ synthesis protein III (pqqIII) (Acinetobacter calcoaceticus)		25.4	48.6	211		
HI0970	1024963	1025817	DHNA synthase (menB) (Escherichia coli)		86.7	95.1	285		
HI1442	1525823	1526707	lamesyldiphosphate synthase (ispA) (Escherichia coli)		53.6	71.2	297		
HI0195	206694	208049	o-succinylbenzoate-CoA synthase (menE) (Escherichia coli)		46.0	66.8	426		
	Heme, porphyrin								
HI1163	1229908	1228940	ferrochelatase (visA) (Escherichia coli)		51.6	69.4	315		
HI0113	119848	122079	heme utilization protein (hxC) (Haemophilus influenzae)		26.4	46.1	695		

76.9

	Surface polysaccharides, lipopolysaccharides & antigens								
HI1563	1628153	1627302	2-dehydro-3-deoxyphosphoactonate aldolase (kdsA) [Escherichia coli]			81.3	91.5	283	
HI0654	696743	695463	3-deoxy-d-manno-octulosonic-acid transferase (kdtA) [Escherichia coli]			50.7	69.9	420	
HI1108	1169176	1168139	ADP-heptose-1ps heptosyltransferase II (rfaF) [Escherichia coli]			63.6	78.9	345	
HI1117	1181141	1180218	ADP-L-glycero-D-mannoheptose-6-epimerase (rfad) [Escherichia coli]			78.2	87.7	308	
HI0058	59659	58898	CTP:CMP-3-deoxy-D-manno-octulosonate-cytidylyl-transferase (kdsB) [Escherichia coli]			65.0	81.7	245	
HI0917	970233	969211	firA protein (firA) [Pasteurella multocida]			84.9	91.1	338	
HI0870	919974	920723	glycosyl transferase (lgtD) [Neisseria gonorrhoeae]			30.3	55.3	200	
HI1584	1646090	1647058	glycosyl transferase (lgtD) [Neisseria gonorrhoeae]			47.3	64.0	328	
HI0653	695463	694996	KDTB protein (kdtB) [Escherichia coli]			52.3	75.8	153	
HI1684	1746281	1747291	kpsF protein (kpsF) [Escherichia coli]			49.3	70.6	294	
HI1543	1607986	1608967	lic-1 operon protein (licA) [Haemophilus influenzae]			99.1	100.0	321	
HI1544	1608970	1609885	lic-1 operon protein (licB) [Haemophilus influenzae]			99.0	99.3	303	
HI1545	1609845	1610543	lic-1 operon protein (licC) [Haemophilus influenzae]			96.5	99.5	198	
HI1546	1610546	1611340	lic-1 operon protein (licD) [Haemophilus influenzae]			88.7	94.0	268	
HI1062	1125450	1124254	lipid A disaccharide synthetase (lpxB) [Escherichia coli]			63.2	77.3	382	
HI0552	571001	570096	lipooligosaccharide biosynthesis protein [Haemophilus influenzae]			98.3	99.0	298	
HI0767	827911	828756	lipooligosaccharide biosynthesis protein [Haemophilus influenzae]			36.4	59.5	267	
HI0869	918779	919990	lsg locus hypothetical protein (GB:M94855_1) [Haemophilus influenzae]			60.5	82.5	400	
HI1706	1770127	1768916	lsg locus hypothetical protein (GB:M94855_1) [Haemophilus influenzae]			99.3	100.0	401	
HI1705	1768916	1768005	lsg locus hypothetical protein (GB:M94855_2) [Haemophilus influenzae]			98.4	98.7	304	
HI1704	1768000	1767322	lsg locus hypothetical protein (GB:M94855_3) [Haemophilus influenzae]			96.0	97.4	226	
HI1703	1766957	1766157	lsg locus hypothetical protein (GB:M94855_4) [Haemophilus influenzae]			96.1	98.4	257	
HI1702	1766142	1765261	lsg locus hypothetical protein (GB:M94855_5) [Haemophilus influenzae]			96.9	98.3	294	
HI1701	1765256	1764456	lsg locus hypothetical protein (GB:M94855_6) [Haemophilus influenzae]			98.9	99.3	267	

76.11

Murein sacculus, peptidoglycan		carboxy-terminal protease, penicillin-binding protein 3 (prc) (Escherichia coli)					
HI1674	1737564	1735481					660
HI1143	1208355	1209272	D-alanine-D-alanine ligase (ddlB) (Escherichia coli)				303
HI1333	1408286	1406850	D-alanyl-D-alanine carboxypeptidase (dacB) (Escherichia coli)				454
HI0066	68323	69618	N-acetylmuramoyl-L-alanine amidase (amiB) (Escherichia coli)				221
HI0383	401990	401532	PC protein (15kd peptidoglycan-associated outer membrane lipoprotein) (pal) (Haemophilus influenzae)				153
HI1731	1795566	1797908	penicillin-binding protein 1B (ponB) (Escherichia coli)				767
HI0032	34810	32858	penicillin-binding protein 2 (pbp2) (Escherichia coli)				609
HI0029	30819	29641	penicillin-binding protein 5 (dacA) (Escherichia coli)				362
HI0198	212582	213439	penicillin-insensitive murein endopeptidase (mepA) (Escherichia coli)				269
HI1138	1201927	1203006	phospho-N-acetylmuramoyl-pentapeptide-transferase E (mraY) (Escherichia coli)				360
HI0038	40689	41741	rod shape-determining protein (mreC) (Escherichia coli)				293
HI0031	32865	31753	rod shape-determining protein (mreB) (Escherichia coli)				358
HI0037	39473	40606	rod shape-determining protein (mreB) (Escherichia coli)				347
HI0039	41744	42229	rod shape-determining protein (mreD) (Escherichia coli)				154
HI0831	878792	880570	soluble lytic murein transglycosylase (slt) (Escherichia coli)				378
HI1141	1205663	1206715	transferase, peptidoglycan synthesis (murG) (Escherichia coli)				350
HI1137	1200560	1201930	UDP-murac-pentapeptide synthetase (murF) (Escherichia coli)				452
HI1136	1199080	1200543	UDP-MurNac-tripeptide synthetase (murE) (Escherichia coli)				463
HI0270	301245	302267	UDP-N-acetylenolpyruvoylglucosamine reductase (murB) (Escherichia coli)				340
HI1083	1148434	1147163	UDP-N-acetylglucosamine enolpyruvyl transferase (murZ) (Escherichia coli)				419
HI1142	1206856	1208280	UDP-N-acetylmuramate-alanine ligase (murC) (Escherichia coli)				470
HI1139	1203132	1204442	UDP-N-acetylmuramoylalanine-D-glutamate ligase (murD) (Escherichia coli)				437
HI1499	1569479	1569826	N-acetylmuramoyl-L-alanine amidase (Bacteriophage T3)				97

76.13

Amino sugars						
HI0431	452989	451160	glutamine amidotransferase (glmS) [Escherichia coli]		72.1	84.3
HI0141	155859	154717	N-acetylglucosamine-6-phosphate deacetylase (nagA) [Escherichia coli]		54.5	72.1
HI0142	156944	156135	nagB protein (nagB) [Escherichia coli]		74.2	88.1
						260
Other						
HI0048	49257	48403	7-alpha-hydroxysteroid dehydrogenase (hdhA) [Escherichia coli]		32.4	55.1
HI1207	1271536	1270334	acetate kinase (ackA) [Escherichia coli]		69.1	83.9
HI0951	1009728	1008367	GABA transaminase (gabT) [Escherichia coli]		34.4	55.8
HI0111	118858	119484	glutathione transferase (bphH) [Pseudomonas sp.]		37.6	57.4
HI0693	734488	735996	glycerol kinase (glpK) [Escherichia coli]		76.9	89.2
HI0586	606429	605161	hippuricase (hipO) [Campylobacter jejuni]		27.8	49.6
HI0543	564874	564575	urease (ureA) [Helicobacter heilmannii]		62.4	76.2
HI0539	561668	561087	urease accessory protein (UreF) [Bacillus sp.]		31.8	54.9
HI0541	564179	562464	urease alpha subunit (urea amidohydrolase) (ureC) [Bacillus sp.]		67.3	82.1
HI0540	562333	561779	urease protein (ureE) [Helicobacter pylori]		31.0	56.8
HI0538	560981	560307	urease protein (ureG) [Helicobacter pylori]		70.7	86.9
HI0537	560229	559447	urease protein (ureH) [Helicobacter pylori]		31.5	53.9
HI0542	564180	564574	urease subunit B (ureB) [Escherichia coli]		61.8	77.5
						103
Energy metabolism						
Amino acids, amines						
HI0536	559266	557842	aspartase (aspA) [Escherichia coli]		78.2	89.1
HI0597	617739	616810	carbamate kinase (arcC) [Pseudomonas aeruginosa]		78.3	87.7
HI0747	802651	803697	L-asparaginase II (ansB) [Escherichia coli]		70.5	81.2
HI0290	323270	321907	L-serine deaminase (sdaA) [Escherichia coli]		68.6	83.3
						454

	Sugars								
HI0820	869307	868288	aldose 1-epimerase precursor (mularotase) (mro) (Acinetobacter calcoaceticus)	36.8	54.7	326			
HI0055	55016	56197	D-mannonate hydrolase (uxuA) (Escherichia coli)	72.8	85.8	394			
HI1119	1181808	1182476	deoxyribose aldolase (deoC) (Mycoplasma hominis)	49.0	68.5	200			
HI0615	644708	643299	fucokinase (fucK) (Escherichia coli)	41.1	64.5	459			
HI0613	642828	642181	fucose-1-phosphate aldolase (fucA) (Escherichia coli)	64.7	81.4	215			
HI1014	1075981	1076610	fucose-1-phosphate aldolase (fucA) (Escherichia coli)	32.9	51.8	163			
HI0821	870510	869320	galactokinase (galK) (Haemophilus influenzae)	98.4	99.0	384			
HI0145	159883	158984	glucose kinase (glk) (Streptomyces coelicolor)	33.6	53.2	303			
HI0616	646595	644784	L-fucose isomerase (fucI) (Escherichia coli)	69.5	84.5	583			
HI1027	1090247	1089519	L-ribulose-phosphate 4-epimerase (araD) (Escherichia coli)	72.3	81.8	231			
HI1111	1173107	1171938	mal inducer biosynthesis blocker (malY) (Escherichia coli)	28.1	51.6	375			
HI0143	158111	157233	N-acetylneuraminase lyase (nanA) (Escherichia coli)	36.2	61.4	291			
HI0507	521330	522247	ribokinase (rbsK) (Escherichia coli)	56.0	74.8	302			
HI1115	1177307	1178623	xylose isomerase (xylA) (Escherichia coli)	71.3	87.2	439			
HI1116	1178629	1180161	xylulose kinase (xylulokinase) (Escherichia coli)	33.1	50.0	479			
	Glycolysis								
HI0449	470280	469342	1-phosphofructokinase (fruK) (Escherichia coli)	55.4	74.1	304			
HI0984	1039579	1038617	6-phosphofructokinase (pfkA) (Escherichia coli)	74.4	84.4	319			
HI0934	990636	989329	enolase (eno) (Bacillus subtilis)	65.9	78.5	413			
HI0526	547668	546592	fructose-bisphosphate aldolase (fba) (Escherichia coli)	71.3	85.8	359			
HI1582	1643750	1645438	glucose-6-phosphate isomerase (pgi) (Escherichia coli)	76.9	88.7	548			
HI0001	1	600	glyceraldehyde-3-phosphate dehydrogenase (gapdH) (Escherichia coli)	85.8	90.3	133			
HI0527	548939	547782	phosphoglycerate kinase (pgk) (Escherichia coli)	81.1	90.7	387			
HI0759	820852	821533	phosphoglyceromutase (gpmA) (Zymomonas mobilis)	58.9	74.6	222			
HI1579	1639619	1641052	pyruvate kinase type II (pykA) (Escherichia coli)	77.2	87.5	480			
HI0680	719664	720452	triosephosphate isomerase (tpiA) (Escherichia coli)	74.4	80.7	253			



Pyruvate dehydrogenase									
HI1235	1303195	1301495	dihydrolipoamide acetyltransferase (aceF) (Escherichia coli)			72.8	82.4	526	
HI0194	206108	205248	dihydrolipoamide acetyltransferase (acoC) (Pseudomonas putida)			27.8	49.1	235	
HI1234	1301378	1299945	lipoamide dehydrogenase (lpdA) (Escherichia coli)			81.5	91.6	474	
HI1236	1305918	1303261	pyruvate dehydrogenase (aceE) (Escherichia coli)			68.6	84.0	886	
	TCA cycle								
HI1668	1731748	1728899	2-oxoglutarate dehydrogenase (sucA) (Escherichia coli)			69.0	80.7	930	
HI0025	27397	26393	acetate:SH-citrate lyase ligase (AMP) (Klebsiella pneumoniae)			48.9	68.4	321	
HI0022	25179	23680	citrate lyase alpha chain (acyl lyase subunit) (citF) (Klebsiella pneumoniae)			72.1	86.1	469	
HI0023	26068	25457	citrate lyase beta chain (acyl lyase subunit) (Klebsiella pneumoniae)			62.3	81.9	203	
HI0024	26352	26068	citrate lyase gamma chain (acyl lyase subunit) (citD) (Klebsiella pneumoniae)			52.1	71.9	97	
HI1667	1728793	1727567	dihydrolipoamide succinyltransferase (sucB) (Escherichia coli)			73.6	84.5	403	
HI1403	1493925	1495316	fumarate hydratase class II (fumarase) (fumC) (Escherichia coli)			61.8	74.2	460	
HI1213	1275907	1276839	malate dehydrogenase (mdh) (Escherichia coli)			78.5	85.1	303	
HI1248	1317431	1319698	malic acid enzyme (Bacillus stearothermophilus)			49.5	68.3	376	
HI1200	1262687	1263565	succinyl-CoA synthetase alpha-subunit (sucD) (Escherichia coli)			83.4	91.7	289	
HI1199	1261518	1262684	succinyl-CoA synthetase beta-subunit (sucC) (Escherichia coli)			64.7	80.2	388	
	Pentose phosphate pathway								
HI0555	574159	572708	6-phosphogluconate dehydrogenase, decarboxylating (gnd) (Escherichia coli)			54.0	71.1	464	
HI0560	577777	576296	glucose-6-phosphate 1-dehydrogenase (G6PD) (Synechococcus sp.)			46.2	65.3	483	

HI1025	1088660	1086666	transketolase 1 (TK 1) (tklA) (Escherichia coli)	77.1	87.5	664
	Entner-Doudoroff					
HI0047	48381	47746	2-keto-3-deoxy-6-phosphogluconate aldolase (eda) (Escherichia coli)	37.3	63.2	193
HI0049	50201	49260	2-keto-3-deoxy-D-gluconate kinase (kdgK) (Erwinia chrysanthemi)	44.2	64.5	300
	Aerobic					
HI1655	1715678	1713987	D-lactate dehydrogenase (dld) (Escherichia coli)	59.5	77.7	560
HI1166	1234330	1231250	D-lactate dehydrogenase (dld) (Saccharomyces cerevisiae)	27.6	47.7	427
HI0607	635168	636172	glycerol-3-phosphate dehydrogenase (gpsA) (Escherichia coli)	66.6	81.5	335
HI0749	805382	806713	NADH dehydrogenase (ndh) (Escherichia coli)	57.8	75.4	430
	Anaerobic					
HI1049	1112944	1110527	anaerobic dimethyl sulfoxide reductase A (dmsA) (Escherichia coli)	74.0	86.3	785
HI1048	1110513	1109899	anaerobic dimethyl sulfoxide reductase B (dmsB) (Escherichia coli)	72.1	84.8	204
HI1047	1109894	1109058	anaerobic dimethyl sulfoxide reductase C (dmsC) (Escherichia coli)	41.0	65.0	287
HI0646	688485	687382	cytochrome C-type protein (lorC) (Escherichia coli)	37.4	54.7	365
HI0350	374535	375134	denitrification system component (nirT) (Pseudomonas stutzeri)	51.7	71.6	176
HI0009	9878	10783	ldhE protein (ldhE) (Escherichia coli)	50.8	71.6	307
HI0006	5067	8156	formate dehydrogenase, nitrate-inducible major subunit (ldnG) (Escherichia coli)	64.4	79.2	1016
HI0005	4802	3993	formate dehydrogenase-N affector (ldhD) (Escherichia coli)	57.7	71.0	249
HI0008	9035	9805	formate dehydrogenase-O gamma subunit (ldol) (Escherichia coli)	52.8	72.1	195
HI0007	8161	9096	formate dehydrogenase-O, beta subunit (ldoH) (Escherichia coli)	72.2	85.6	297
HI1071	1133439	1131826	formate-dependent nitrite reductase (cytochrome C552) (nrfA) (Escherichia coli)	56.7	75.3	450
HI1070	1131779	1131102	formate-dependent nitrite reductase (nrfB) (Escherichia coli)	50.0	66.9	134

HI1069	1131102	1130428	formate-dependent nitrite reductase protein Fe-S centers (nrdC) (Escherichia coli)	64.2	81.2	217
HI1068	1130428	1129466	formate-dependent nitrite reductase transmembrane protein (nrdD) (Escherichia coli)	48.2	68.4	312
HI0835	882094	882529	fumarate reductase (frdC) (Escherichia coli)	49.2	72.3	129
HI0834	882093	881752	fumarate reductase 13 kDa hydrophobic protein (frdD) (Escherichia coli)	53.0	76.5	119
HI0837	885089	883293	fumarate reductase, flavoprotein subunit (frdA) (Escherichia coli)	75.4	87.2	602
HI0836	883357	882530	fumarate reductase, iron-sulfur protein (frdB) (Escherichia coli)	75.5	85.3	244
HI0681	720855	720541	glpE protein (glpE) (Escherichia coli)	43.3	63.5	103
HI0620	651184	651759	glpG protein (glpG) (Escherichia coli)	39.1	64.8	178
HI0687	729180	727492	glycerol-3-phosphate dehydrogenase, subunit A (glpA) (Escherichia coli)	69.9	82.7	531
HI0686	727529	726204	glycerol-3-phosphate dehydrogenase, subunit B (glpB) (Escherichia coli)	42.3	60.3	414
HI0685	726189	724912	glycerol-3-phosphate dehydrogenase, subunit C (glpC) (Escherichia coli)	58.8	76.0	393
HI1395	1487087	1487358	hydrogenase isoenzymes formation protein (hypC) (Escherichia coli)	63.2	81.6	76
Electron transport						
HI0887	936816	938552	C-type cytochrome biogenesis protein (copper tolerance) (cycZ) (Escherichia coli)	48.8	67.7	557
HI1078	1141318	1139756	cytochrome oxidase d subunit I (cydA) (Escherichia coli)	64.3	82.4	515
HI1077	1139738	1138605	cytochrome oxidase d subunit II (cydB) (Escherichia coli)	60.9	78.4	379
HI0529	549872	550341	ferredoxin (ldx) (Chromatium vinosum)	59.5	77.2	78
HI0374	394564	394226	ferredoxin (ldx) (Escherichia coli)	64.5	83.6	110
HI0192	205148	204627	flavodoxin (lldA) (Escherichia coli)	76.9	87.3	173
HI1365	1446272	1447807	NAD(P) transhydrogenase subunit alpha (pntA) (Escherichia coli)	73.7	84.1	509
HI1366	1447821	1449242	NAD(P) transhydrogenase subunit beta (pntB) (Escherichia coli)	80.5	87.7	462
HI1281	1355273	1354614	NAD(P)H-flavin oxidoreductase (Vibrio fischeri)	33.3	54.8	211
Fermentation						

[illegible]

Fatty acid/phospholipid metabolism							
HI0773	834230	832896	acetyl coenzyme A acetyltransferase (thiolase) (fadA) (Clostridium acetobutylicum)	63.0	80.4	391	
HI0428	448891	448169	fadR protein involved in fatty acid metabolism (fadR) (Escherichia coli)	47.4	68.4	234	
HI1064	1126738	1126295	(3R)-hydroxymyristol acyl carrier protein dehydrase (fabZ) (Escherichia coli)	68.1	85.1	141	
HI0156	171552	170827	3-ketoacyl-acyl carrier protein reductase (fabG) (Escherichia coli)	73.4	88.4	241	
HI0408	427385	426441	acetyl-CoA carboxylase (accA) (Escherichia coli)	75.3	88.3	318	
HI0155	170568	170341	acyl carrier protein (acpP) (Escherichia coli)	82.7	90.7	75	
HI0076	82175	83032	acyl-CoA thioesterase II (tesB) (Escherichia coli)	52.3	73.1	283	
HI1539	1605754	1604537	beta-ketoacyl-ACP synthase I (fabB) (Escherichia coli)	72.8	83.7	403	
HI0158	174085	173138	beta-ketoacyl-acyl carrier protein synthase III (fabH) (Escherichia coli)	65.9	79.8	317	
HI0973	1027538	1028002	biotin carboxyl carrier protein (accB) (Escherichia coli)	71.2	82.7	156	
HI0974	1028180	1029523	biotin carboxylase (accC) (Escherichia coli)	81.5	91.3	448	
HI1328	1404041	1404571	D-3-hydroxydecanoyl-(acyl carrier-protein) dehydratase (fabA) (Escherichia coli)	79.2	91.7	168	
HI0337	362881	363234	diacylglycerol kinase (dgkA) (Escherichia coli)	50.9	71.8	110	
HI0002	601	2421	long chain fatty acid coA ligase (Homo sapiens)	29.5	52.8	575	
HI0157	172507	171572	malonyl coenzyme A-acyl carrier protein transacylase (fabD) (Escherichia coli)	71.0	81.6	309	
HI1740	1811556	1810672	short chain alcohol dehydrogenase homolog (envM) (Escherichia coli)	75.3	84.9	259	
HI1438	1521691	1520741	USG-1 protein (usg) (Escherichia coli)	32.7	53.9	334	
HI0736	788371	787652	1-acyl-glycerol-3-phosphate acyltransferase (plsC) (Escherichia coli)	62.2	78.2	238	
HI0921	975561	974698	CDP-diglyceride synthetase (cdsA) (Escherichia coli)	48.4	66.5	246	
HI0750	809228	806799	glycerol-3-phosphate acyltransferase (plsB) (Escherichia coli)	57.3	75.7	804	
HI0212	225946	225224	phosphatidylglycerophosphate phosphatase B (pgpB) (Escherichia coli)	35.7	60.3	220	
HI0123	138207	138761	phosphatidylglycerophosphate synthase (pgsA) (Escherichia coli)	66.5	83.0	182	

HI0161	175145	176014	phosphatidylserine decarboxylase proenzyme (psd) (Escherichia coli)	57.6	75.5	280
HI0427	446754	448118	phosphatidylserine synthase (pssA) (Escherichia coli)	49.2	70.8	452
HI0691	732349	733440	protein D (hpd) (Haemophilus influenzae)	98.4	99.2	364
Purin s, pyrimidines, nucleosides and nucleotides						
Purine ribonucleotide biosynthesis						
HI1622	1682920	1684005	5'-phosphoribosyl-5-amino-4-imidazole carboxylase II (purK) (Escherichia coli)	56.8	71.9	351
HI1434	1517646	1516615	5'-phosphoribosyl-5-aminoimidazole synthetase (purM) (Escherichia coli)	76.5	86.7	344
HI1749	1829283	1828660	5'guanylate kinase (gmK) (Escherichia coli)	64.7	81.6	206
HI0351	375941	375300	adenylate kinase (ATP-AMP transphosphorylase) (adk) (Haemophilus influenzae)	99.5	99.5	214
HI0641	679574	681094	adenylosuccinate lyase (purB) (Escherichia coli)	76.5	87.9	456
HI1639	1694462	1695757	adenylosuccinate synthetase (purA) (Escherichia coli)	75.7	87.3	432
HI1210	1272783	1274297	amidophosphoribosyltransferase (purF) (Escherichia coli)	69.1	84.0	504
HI0754	812369	816328	formylglycineamide ribonucleotide synthetase (purL) (Escherichia coli)	69.7	82.0	1290
HI1594	1655627	1656460	formyltetrahydrofolate hydrolase (purU) (Escherichia coli)	72.6	85.2	277
HI0223	250532	252100	guaA protein (guaA) (Escherichia coli)	78.1	87.6	525
HI0222	248355	249818	inosine-5'-monophosphate dehydrogenase (guaB) (Acinetobacter calcoaceticus)	62.7	80.9	487
HI0878	928811	929233	nucleoside diphosphate kinase (ndk) (Escherichia coli)	63.0	73.9	138
HI0890	940953	942239	phosphoribosylamine-glycine ligase (purD) (Escherichia coli)	75.2	84.5	427
HI1621	1682355	1682847	phosphoribosylaminoimidazole carboxylase catalytic subunit (purE) (Haemophilus influenzae)	94.4	96.9	161
HI0889	939259	940854	phosphoribosylaminoimidazolecarboxamide formyltransferase (purH) (Escherichia coli)	77.2	86.5	525
HI1433	1516557	1515922	phosphoribosylglycinamide formyltransferase (purN) (Escherichia coli)	51.9	71.4	210
HI1615	1674317	1675261	phosphoribosylpyrophosphate synthetase (prsA) (Salmonella typhimurium)	84.1	91.1	314
HI1732	1798036	1798953	SAICAR synthetase (purC) (Streptococcus pneumoniae)	29.8	54.8	204

Pyrimidine ribonucleotide biosyn						
HI1406	1497997	1496981	dihydroorotate dehydrogenase (dihydroorotate oxidase) (pyrD) (Escherichia coli)	60.7	77.4	334
HI0274	305799	305161	orotate phosphoribosyltransferase (pyrE) (Escherichia coli)	69.0	83.6	213
HI1228	1293965	1294282	pyrF operon encoding orotidine 5'-monophosphate (OMP) decarboxylase (Escherichia coli)	77.1	87.6	105
HI1227	1293266	1293955	pyrF protein (pyrF) (Escherichia coli)	62.3	79.4	228
HI0461	480053	479517	uracil phosphoribosyltransferase (pyrR) (Bacillus caldolyticus)	52.2	73.9	179
2'-deoxyribonucleotide metabolism						
HI0075	79934	82054	anaerobic ribonucleoside-triphosphate reductase (nrdD) (Escherichia coli)	77.4	88.2	702
HI0133	146656	147240	deoxycytidine triphosphate deaminase (dcd) (Escherichia coli)	75.6	86.5	193
HI0956	1012787	1013239	deoxyuridine triphosphate (dut) (Escherichia coli)	75.5	90.7	151
HI1538	1604204	1604464	glutaredoxin (grx) (Escherichia coli)	69.9	79.5	83
HI1666	1726318	1727445	nrdB protein (nrdB) (Escherichia coli)	85.4	92.6	376
HI1665	1723831	1726173	ribonucleoside-diphosphate reductase 1 alpha chain (nrdA) (Escherichia coli)	83.4	92.2	761
HI1161	1227925	1226972	thioredoxin reductase (trxB) (Escherichia coli)	75.9	85.8	316
HI0907	958914	959762	thymidylate synthetase (thyA) (Escherichia coli)	35.3	55.0	264
Salvage of nucleosides and nucleotides						
HI0585	605064	603094	2',3'-cyclic-nucleotide 2'-phosphodiesterase (cpdB) (Escherichia coli)	62.4	77.7	641
HI1233	1299794	1299255	adenine phosphoribosyltransferase (apt) (Escherichia coli)	66.1	83.1	177

HI0553	571120	571943	adenosine-tetraphosphatase (apaH) (Escherichia coli)	52.4	73.1	271
HI1353	1426390	1427265	cytidine deaminase (cytidine aminohydrolase) (cda) (Escherichia coli)	50.0	63.4	253
HI1222	1288579	1289628	cytidylate kinase (cmk) (Escherichia coli)	64.5	79.3	217
HI1652	1711636	1710842	cytidylate kinase (cmk) (Escherichia coli)	63.5	76.8	202
HI0520	540879	540166	purine-nucleoside phosphorylase (deoD) (Escherichia coli)	84.3	90.2	235
HI0531	552177	551599	thymidine kinase (tdk) (Escherichia coli)	68.6	82.4	188
HI1231	1297050	1296427	uracil phosphoribosyltransferase (upp) (Escherichia coli)	83.2	93.8	208
HI0282	312879	313655	uridine phosphorylase (udp) (Escherichia coli)	72.0	84.8	250
HI0676	716559	716095	xanthine guanine phosphoribosyl transferase gpt (xgprt) (Escherichia coli)	72.1	87.7	152
HI0694	736541	736077	xanthine-guanine phosphoribosyltransferase (xgprt) (Salmonella typhimurium)	74.0	87.7	152
HI1280	1353404	1354561	putative ATPase (mrp) (Escherichia coli)	66.0	79.0	353
			Sugar-nucleotide biosynthesis, conversions			
HI0207	219511	221319	5'-nucleotidase (ushA) (Homo sapiens)	34.5	54.8	487
HI1282	1355378	1356061	CMP-NeuNAc synthetase (siaB) (Neisseria meningitidis)	47.1	64.3	221
HI0822	871597	870551	galactose-1-phosphate uridylyltransferase (galT) (Haemophilus influenzae)	99.1	100.0	349
HI0814	862632	861748	glucosephosphate uridylyltransferase (galU) (Escherichia coli)	74.0	86.1	287
HI0353	378461	377448	udp-glucose 4-epimerase (galactowaldenase) (galE) (Haemophilus influenzae)	99.1	99.1	338
HI0644	682446	683813	UDP-N-acetylglucosamine pyrophosphorylase (glmU) (Escherichia coli)	68.6	83.1	456
			Nucleotide and nucleoside interconversions			
HI1302	1376759	1378139	deoxyguanosine triphosphate triphosphohydrolase (dgt) (Escherichia coli)	38.2	57.6	469
HI1079	1141970	1143603	pyrG protein (pyrG) (Escherichia coli)	80.4	90.5	545
HI0132	146006	146644	uridine kinase (uridine monophosphokinase) (udk) (Escherichia coli)	67.8	84.7	202



R gulatory functions							
HI0606	632563	635091	adenylate cyclase (cyaA) (Haemophilus influenzae)		100.0	100.0	843
HI0886	936624	935917	aerobic respiration control protein ARCA (DYE resistance protein) (arcA) (Escherichia coli)		77.2	87.8	237
HI0221	238723	248354	aerobic respiration control sensor protein (arcB) (Escherichia coli)		45.7	70.4	768
HI1054	1117872	1116979	araC-like transcription regulator (Streptomyces lividans)		25.7	47.7	303
HI1212	1275700	1275248	arginine repressor protein (argR) (Escherichia coli)		69.1	81.2	149
HI0237	265657	265310	arsC protein (arsC) (Plasmid R773)		38.3	56.5	114
HI0464	482094	484502	ATP-dependent proteinase (lon) (Escherichia coli)		74.5	87.9	769
HI0336	360636	362863	ATP:GTP 3'-pyrophosphotransferase (relA) (Escherichia coli)		62.9	80.5	741
HI1130	1193658	1195126	carbon starvation protein (cstA) (Escherichia coli)		32.1	53.5	499
HI0815	862845	862657	carbon storage regulator (cstA) (Escherichia coli)		68.4	91.2	57
HI0806	853619	853063	cyclic AMP receptor protein (crp) (Haemophilus influenzae)		27.2	46.7	174
HI0959	1014161	1014832	cyclic AMP receptor protein (crp) (Haemophilus influenzae)		100.0	100.0	224
HI1203	1265444	1266412	cys regulon transcriptional activator (cysB) (Escherichia coli)		63.3	79.3	324
HI0191	204595	204158	feric uptake regulation protein (fur) (Escherichia coli)		61.4	75.0	139
HI1457	1537858	1537391	fimbrial transcription regulation repressor (pilB) (Neisseria gonorrhoeae)		32.3	53.2	124
HI1459	1539614	1538556	fimbrial transcription regulation repressor (pilB) (Neisseria gonorrhoeae)		59.0	72.6	325
HI1263	1336661	1337548	folypolyglutamate-dihydrofolate synthetase expression regulator (accD) (Escherichia coli)		69.5	82.5	290
HI1430	1512975	1513745	fumarate (and nitrate) reduction regulatory protein (fnr) (Escherichia coli)		78.8	88.8	240
HI0823	871805	872800	galactose operon repressor (galS) (Haemophilus influenzae)		99.1	99.4	332
HI0756	817661	818569	glucokinase regulator (Rattus norvegicus)		31.8	56.1	512

HI0621	651792	652556	glycerol-3-phosphate regulon repressor (glpR) (Escherichia coli)	61.5	77.4	252
HI1011	1073676	1073047	glycerol-3-phosphate regulon repressor (glpR) (Escherichia coli)	28.6	50.3	198
HI1197	1259493	1260395	glycine cleavage system transcriptional activator (gcvA) (Escherichia coli)	51.7	69.1	298
HI0013	13742	12837	GTP-binding protein (era) (Escherichia coli)	77.9	87.0	299
HI0879	930478	929309	GTP-binding protein (obg) (Bacillus subtilis)	47.7	70.9	332
HI0573	592001	591099	hydrogen peroxide-inducible activator (oxyR) (Escherichia coli)	71.1	85.9	298
HI0617	647526	646780	L-lucose operon activator (lucR) (Escherichia coli)	35.1	56.1	229
HI0401	420131	420952	lacZ expression regulator (icc) (Escherichia coli)	52.9	71.3	261
HI0225	253133	253636	leucine responsive regulatory protein (lrp) (Escherichia coli)	29.6	52.6	152
HI1602	1663150	1662653	leucine responsive regulatory protein (lrp) (Escherichia coli)	77.2	86.7	158
HI0751	809477	810103	LEXA repressor (lexA) (Escherichia coli)	68.1	85.3	202
HI1465	1542848	1542810	lipooligosaccharide protein (lex2A) (Haemophilus influenzae)	44.4	66.7	9
HI1466	1542849	1543428	lipooligosaccharide protein (lex2A) (Haemophilus influenzae)	50.0	66.7	48
HI0296	328190	327876	metF aporepressor (metJ) (Escherichia coli)	81.9	93.3	105
HI1478	1558154	1557312	molybdenum transport system alternative nitrogenase regulator (modD) (Rhodobacter capsulatus)	31.8	51.7	259
HI0200	214274	215227	msbB protein (msbB) (Escherichia coli)	45.3	67.0	301
HI0411	429238	430662	msbB protein (msbB) (Escherichia coli)	50.9	69.3	284
HI0712	756824	757117	negative regulator of translation (relB) (Escherichia coli)	28.3	48.3	60
HI0631	667822	668406	negative rpo regulator(mclA) (Escherichia coli)	40.1	62.9	199
HI0269	299532	301232	nitrate sensor protein (narQ) (Escherichia coli)	38.6	63.0	555
HI0728	778003	777380	nitrate/nitrite response regulator protein (narP) (Escherichia coli)	59.6	79.3	205
HI0339	363915	364250	nitrogen regulatory protein P-II (glnB) (Escherichia coli)	77.7	93.8	112
HI1747	1828067	1826037	penta-phosphate guanosine-3'-pyrophosphohydrolase (spoT) (Escherichia coli)	58.8	76.6	675
HI1381	1475017	1473741	phosphate regulon sensor protein (phoR) (Escherichia coli)	41.8	66.8	335
HI1382	1475709	1475017	phosphate regulon transcriptional regulatory protein (phoB) (Escherichia coli)	52.9	71.8	227

HI0765	827030	825768	probable nadAB transcriptional regulator (nadR) [Escherichia coli]	54.6	75.1	349
HI1641	1697003	1698115	purine nucleotide synthesis repressor protein (purR) [Escherichia coli]	55.9	74.5	328
HI0164	178405	178713	putative murein gene regulator (bolA) [Escherichia coli]	47.1	65.7	102
HI0508	522278	523273	rbs repressor (rbsR) [Escherichia coli]	48.8	71.0	329
HI0565	582225	581776	regulatory protein (asnC) [Escherichia coli]	68.0	81.0	147
HI1617	1677452	1676583	regulatory protein sfs1 involved in maltose metabolism (sisA) [Escherichia coli]	54.3	71.2	218
HI0895	946128	946688	repressor for cytochrome P450 (Bm3R1) [Bacillus megaterium]	23.3	50.6	182
HI0271	302396	303238	RNA polymerase sigma-32 factor (heat shock regulatory protein F334) (rpoH) [Escherichia coli]	70.8	86.8	281
HI0535	555646	557532	RNA polymerase sigma-70 factor (rpoD) [Escherichia coli]	68.9	80.8	608
HI0630	667228	667794	RNA polymerase sigma-E factor (rpoE) [Escherichia coli]	73.0	87.8	189
HI1713	1781137	1779785	sensory protein for basR (basS) [Escherichia coli]	30.0	55.7	253
HI1444	1529117	1528668	stringent starvation protein (sspB) [Escherichia coli]	63.2	81.1	106
HI1445	1529755	1529120	stringent starvation protein A (ssaA) [Haemophilus somnus]	76.9	87.3	212
HI1745	1815630	1814704	trans-activator of metE and meth (metR) [Escherichia coli]	39.5	60.8	294
HI0360	382477	383121	transcription activator (tenA) [Bacillus subtilis]	27.8	48.3	208
HI0683	722643	721768	transcriptional activator protein (ilvY) [Escherichia coli]	47.4	70.3	293
HI1714	1781799	1781137	transcriptional regulatory protein (basR) [Escherichia coli]	43.5	59.7	216
HI0412	430780	431733	transcriptional regulatory protein (tyrR) [Escherichia coli]	48.2	66.8	306
HI0832	880611	880913	tryptophan repressor (ltpR) [Enterobacter aerogenes]	39.8	67.0	88
HI0054	54188	54985	uxu operon regulator (uxuR) [Escherichia coli]	50.0	72.1	246
HI1109	1170415	1169255	xylose operon regulatory protein (xylR) [Escherichia coli]	57.3	75.3	384
Replication						
DNA - replication, restr/modification, recombination						

HI0761	822003	823136	A/G-specific adenine glycosylase (mutY) [Escherichia coli]	61.6	75.1	341
HI0995	1056674	1055313	chromosomal replication initiator protein (dnaA) [Escherichia coli]	61.7	79.7	464
HI1229	1294415	1294317	chromosomal replication initiator protein (dnaA) [Escherichia coli]	50.0	75.0	12
HI0316	345720	345151	crossover junction endodeoxyribonuclease (ruvC) [Escherichia coli]	78.5	88.3	163
HI0955	1011537	1012736	dif protein (dif) [Escherichia coli]	61.1	76.8	402
HI0210	223259	224116	DNA adenine methylase (dam) [Escherichia coli]	55.4	71.4	266
HI1267	1343755	1341116	DNA gyrase, subunit A (gyrA) [Escherichia coli]	70.6	84.9	859
HI0569	587397	584980	DNA gyrase, subunit B (gyrB) [Escherichia coli]	74.7	85.9	803
HI1191	1255302	1253122	DNA helicase II (uvrD) [Haemophilus influenzae]	96.8	97.5	727
HI1102	1162989	1160953	DNA ligase (lig) [Escherichia coli]	63.7	79.9	666
HI0405	423539	424207	DNA mismatch protein (mutH) [Escherichia coli]	60.4	80.7	212
HI0709	750565	753147	DNA mismatch repair protein (mutS) [Escherichia coli]	71.0	84.0	853
HI0067	69622	71508	DNA mismatch repair protein MUTL (mutL) [Escherichia coli]	50.2	67.3	612
HI0858	904919	902130	DNA polymerase I (polA) [Escherichia coli]	63.1	77.0	928
HI0994	1055297	1054200	DNA polymerase III beta-subunit (dnaN) [Escherichia coli]	62.6	80.3	366
HI0457	476761	475763	DNA polymerase III delta prime subunit (holB) [Escherichia coli]	35.3	57.4	316
HI0925	979730	980761	DNA polymerase III delta subunit (holA) [Escherichia coli]	45.2	62.0	332
HI0138	152669	151902	DNA polymerase III epsilon subunit (dnaQ) [Escherichia coli]	61.3	76.5	236
HI0741	799019	795544	DNA polymerase III, alpha chain (dnaE) [Escherichia coli]	71.9	85.7	1159
HI1402	1493690	1493259	DNA polymerase III, chi subunit (holC) [Haemophilus influenzae]	98.9	98.9	88
HI0011	11672	11271	DNA polymerase III, psi subunit (holD) [Escherichia coli]	34.4	59.2	123
HI0534	553659	555645	DNA primase (dnaG) [Escherichia coli]	56.5	73.8	571
HI1746	1826037	1823959	DNA recombinase (recG) [Escherichia coli]	66.5	80.1	693
HI0070	77166	75493	DNA repair protein (recN) [Escherichia coli]	48.6	67.3	533
HI0659	699507	700058	DNA topoisomerase I (topA) [Bacillus subtilis]	34.2	55.0	110
HI0656	698124	697570	DNA-3-methyladenine glycosidase I (tagI) [Escherichia coli]	62.6	76.0	179

HI0730	779457	781969	DNA-dependent ATPase, DNA helicase (recQ) (Escherichia coli)	62.9	77.6	589
HI0568	584860	584159	dod protein (dod) (Serratia marcescens)	81.4	93.3	210
HI0062	65230	65664	dosage-dependent dnaK suppressor protein (dksA) (Escherichia coli)	73.9	83.8	142
HI0948	1005798	1004986	formamidopyrimidine-DNA glycosylase (fpg) (Escherichia coli)	57.6	74.7	269
HI0584	602405	600519	glucose inhibited division protein (gidA) (Escherichia coli)	76.1	87.3	627
HI0488	506816	506208	glucose inhibited division protein (gidB) (Escherichia coli)	64.0	78.0	200
HI0982	1037496	1037792	Hin recombinational enhancer binding protein (his) (Escherichia coli)	81.6	92.9	97
HI0514	528338	527565	HincII endonuclease (HincII) (Haemophilus influenzae)	98.4	98.4	258
HI1397	1491189	1490263	HindIII modification methyltransferase (hindIII-M) (Haemophilus influenzae)	99.4	99.4	309
HI1398	1492072	1491173	HindIII restriction endonuclease (hindIII-R) (Haemophilus influenzae)	99.7	99.7	300
HI0315	345085	344474	holliday junction DNA helicase (ruvA) (Escherichia coli)	58.8	79.9	203
HI0314	344463	343459	holliday junction DNA helicase (ruvB) (Escherichia coli)	80.9	90.0	330
HI0678	719064	718180	integrase/recombinase protein (xerC) (Escherichia coli)	58.0	74.4	293
HI1316	1391102	1391389	integration host factor alpha-subunit (himA) (Escherichia coli)	63.8	83.0	94
HI1224	1291400	1291681	integration host factor beta-subunit (IHf-beta) (himD) (Escherichia coli)	56.5	77.2	92
HI0404	422970	423539	methylated-DNA--protein-cysteine methyltransferase (dat1) (Bacillus subtilis)	40.1	61.7	163
HI0671	713369	713806	mioC protein (mioC) (Escherichia coli)	53.5	71.5	144
HI1043	1104813	1105724	modification methylase HgiDI (MHgDI) (Herpetosiphon aurantiacus)	56.4	70.5	297
HI0515	529891	528338	modification methylase HincII (hincII-M) (Haemophilus influenzae)	98.2	98.6	502
HI0912	963611	964312	mutator mutT (AT-GC transversion) (Escherichia coli)	48.8	72.0	125
HI0193	206098	206688	negative modulator of initiation of replication (seqA) (Escherichia coli)	53.1	71.8	177
HI0548	568202	567879	primosomal protein n precursor (priB) (Escherichia coli)	57.4	75.2	101
HI0341	367532	365343	primosomal protein replication factor (priA) (Escherichia coli)	52.3	70.2	729
HI0389	406402	408321	probable ATP-dependent helicase (dinG) (Escherichia coli)	32.2	51.1	680
HI0993	1054243	1053119	recF protein (recF) (Escherichia coli)	57.0	75.8	356
HI0334	358532	359239	recO protein (recO) (Escherichia coli)	64.6	76.5	226
HI0602	621957	620896	recombinase (recA) (Haemophilus influenzae)	100.0	100.0	354
HI0061	64971	62573	recombination protein (rec2) (Haemophilus influenzae)	99.9	99.9	800

HI0445	464118	464717	recR protein (recR) (Escherichia coli)	74.9	88.4	199
HI0601	620735	620358	regulatory protein (recX) (Pseudomonas fluorescens)	28.6	50.4	117
HI0651	694862	692768	rep helicase (rep) (Escherichia coli)	66.9	82.7	669
HI1232	1299240	1297177	replication protein (dnaX) (Escherichia coli)	52.9	69.8	643
HI1580	1641089	1642600	replicative DNA helicase (dnaB) (Escherichia coli)	68.6	82.8	462
HI1042	1103812	1104813	restriction enzyme (hgiDIR) (Herpetosiphon giganteus)	44.2	63.9	350
HI1175	1241423	1242574	S-adenosylmethionine synthetase 2 (metX) (Escherichia coli)	82.3	91.7	383
HI1429	1512463	1511552	shufflon-specific DNA recombinase (rci) (Escherichia coli)	31.1	55.5	259
HI0251	281830	282333	single-stranded DNA binding protein (ssb) (Haemophilus influenzae)	95.8	98.2	168
HI1578	1639113	1638016	site-specific recombinase (rcb) (Escherichia coli)	36.3	57.0	265
HI1368	1450325	1452928	topoisomerase I (topA) (Escherichia coli)	72.0	84.3	865
HI0446	464736	466688	topoisomerase III (topB) (Escherichia coli)	65.9	79.4	645
HI1535	1599641	1601881	topoisomerase IV subunit A (parC) (Escherichia coli)	71.4	85.4	727
HI1534	1597676	1599571	topoisomerase IV subunit B (parE) (Escherichia coli)	76.5	88.6	630
HI1261	1331575	1335011	transcription-repair coupling factor (trcF) (mld) (Escherichia coli)	64.3	82.7	1134
HI0217	232884	234038	type I restriction enzyme <i>ecok</i> specificity protein (hsdS) (Escherichia coli)	36.1	58.6	394
HI0216	231281	232797	type I restriction enzyme ECOR124/3 I M protein (hsdM) (Escherichia coli)	81.2	89.3	512
HI1290	1368549	1367223	type I restriction enzyme ECOR124/3 I M protein (hsdM) (Escherichia coli)	30.4	53.7	332
HI1288	1365756	1362592	type I restriction enzyme ECOR124/3 R protein (hsdR) (Escherichia coli)	30.4	52.7	991
HI1059	1123091	1121205	type III restriction-modification ECOP15 enzyme (mod) (Escherichia coli)	36.5	55.5	384
HI0018	18087	18743	uracil DNA glycosylase (ung) (Escherichia coli)	70.2	79.5	215
HI0311	342051	342941	xprB protein (xerD) (Escherichia coli)	68.9	84.8	296

	Degradation of DNA						
HI1695	1758680	1759312	endonuclease III (nth) [Escherichia coli]		83.4	91.9	211
HI0250	278528	281829	excinuclease ABC subunit A (uvrA) [Escherichia coli]		81.2	91.0	940
HI1250	1323924	1321888	excinuclease ABC subunit B (uvrB) [Escherichia coli]		78.0	87.7	669
HI0057	58893	57067	excinuclease ABC subunit C (uvrC) [Escherichia coli]		65.9	80.0	588
HI1380	1471626	1473044	exodeoxyribonuclease I (sbcB) [Escherichia coli]		57.5	74.9	462
HI1324	1395898	1399530	exodeoxyribonuclease V (recB) [Escherichia coli]		37.1	58.2	1165
HI0944	998895	1002257	exodeoxyribonuclease V (recC) [Escherichia coli]		40.1	61.2	1114
HI1325	1399533	1401452	exodeoxyribonuclease V (recD) [Escherichia coli]		40.0	59.3	570
HI0041	43872	43072	exonuclease III (xthA) [Escherichia coli]		71.9	83.9	267
HI0399	417972	419288	exonuclease VII, large subunit (xseA) [Escherichia coli]		57.8	74.4	437
HI1217	1280795	1282519	single-stranded-DNA-specific exonuclease (recJ) [Escherichia coli]		59.2	77.3	554
Transcription							
RNA synthesis, modification and DNA transcription							
HI0618	647724	650492	ATP-dependent helicase HEPA (hepA) [Escherichia coli]		53.6	73.6	968
HI0424	444751	443435	ATP-dependent RNA helicase (smbB) [Escherichia coli]		39.8	60.9	448
HI0232	260978	262816	ATP-dependent RNA helicase DEAD (dead) [Escherichia coli]		64.0	78.6	613
HI0804	851485	852468	DNA-directed RNA polymerase alpha chain (rpoA) [Escherichia coli]		91.8	97.0	329
HI0517	534212	538870	DNA-directed RNA polymerase beta chain (rpoB) [Salmonella typhimurium]		83.3	91.9	1342
HI0516	534211	529967	DNA-directed RNA polymerase beta' chain (rpoC) [Escherichia coli]		83.0	90.7	1399
HI1307	1383078	1383509	N utilization substance protein B (nusB) [Escherichia coli]		54.9	71.4	133
HI0063	65915	67269	plasmid copy number control protein (pcnB) [Escherichia coli]		55.7	73.4	404
HI0230	257702	259828	polynucleotide phosphorylase (pnp) [Escherichia coli]		74.2	86.7	708
HI0894	944630	945883	putative ATP-dependent RNA helicase (rhlB) [Escherichia coli]		73.9	84.1	410
HI1748	1828594	1828331	RNA polymerase omega subunit (rpoZ) [Escherichia coli]		64.8	76.1	88
HI1463	1542205	1541624	sigma factor (algU) [Pseudomonas aeruginosa]		27.6	48.8	168

HI0719	764847	765401	transcription antitermination protein (nusG) (Escherichia coli)	73.7	84.4	179
HI0571	589932	590405	transcription elongation factor (greB) (Escherichia coli)	61.5	79.5	156
HI1286	1358486	1360006	transcription factor (nusA) (Salmonella typhimurium)	70.8	84.1	499
HI0297	328437	329696	transcription termination factor rho (rho) (Escherichia coli)	87.4	95.2	419
	Degradation of RNA					
HI0219	234848	237923	anticodon nuclease masking-agent (prfD) (Escherichia coli)	72.9	85.6	291
HI1739	1810586	1808610	exonuclease II (RNaseII) (Escherichia coli)	50.8	68.0	588
HI0392	411354	412550	ribonuclease D (md) (Escherichia coli)	41.3	65.5	365
HI0415	433540	436392	ribonuclease E (me) (Escherichia coli)	60.3	72.3	1058
HI0139	152730	153191	ribonuclease H (mh) (Escherichia coli)	64.9	76.0	154
HI1061	1124258	1123668	ribonuclease HII (EC 31264) (RNase HII) (Escherichia coli)	73.7	82.8	185
HI0014	14422	13742	ribonuclease III (mc) (Escherichia coli)	65.3	80.2	221
HI0275	306539	305826	ribonuclease PH (rph) (Escherichia coli)	78.9	87.8	237
HI1001	1063336	1063743	RNase P (mpA) (Escherichia coli)	69.7	80.7	119
HI0326	351726	352412	RNase T (mt) (Escherichia coli)	65.7	80.9	204
Translation						
	Ribosomal proteins - synthesis, modification					
HI0518	539557	538871	ribosomal protein L1 (rpL1) (Escherichia coli)	85.6	93.4	229
HI0642	681369	681857	ribosomal protein L10 (rpL10) (Salmonella typhimurium)	80.5	89.0	165
HI0519	539990	539565	ribosomal protein L11 (rpL11) (Escherichia coli)	86.6	94.4	142
HI0980	1035484	1036371	ribosomal protein L11 methyltransferase (prmA) (Escherichia coli)	69.2	83.2	291
HI1447	1530773	1530348	ribosomal protein L13 (rpL13) (Haemophilus somnus)	94.4	95.8	142
HI0790	844379	844747	ribosomal protein L14 (rpL14) (Escherichia coli)	94.3	98.4	123
HI0799	847996	848427	ribosomal protein L15 (rpL15) (Escherichia coli)	82.6	91.0	144
HI0786	842244	842651	ribosomal protein L16 (rpL16) (Escherichia coli)	89.7	95.6	136
HI0805	852512	852895	ribosomal protein L17 (rpL17) (Escherichia coli)	89.8	92.1	127



HI0796	846938	847288	ribosomal protein L18 (rpL18) (Escherichia coli)	84.6	91.5	117
HI0202	216787	216440	ribosomal protein L19 (rpL19) (Escherichia coli)	89.5	98.2	114
HI0782	840039	840857	ribosomal protein L2 (rpL2) (Escherichia coli)	85.7	93.4	273
HI1323	1395432	1395782	ribosomal protein L20 (rpL20) (Escherichia coli)	94.0	96.6	117
HI0882	932097	931789	ribosomal protein L21 (rpL21) (Escherichia coli)	79.6	86.4	103
HI0784	841173	841502	ribosomal protein L22 (rpL22) (Escherichia coli)	91.8	97.3	110
HI0781	839722	840018	ribosomal protein L23 (rpL23) (Escherichia coli)	71.7	82.8	99
HI0791	844761	845069	ribosomal protein L24 (rpL24) (Escherichia coli)	76.7	86.4	103
HI1636	1692153	1692437	ribosomal protein L25 (rpL25) (Escherichia coli)	61.9	77.4	84
HI0881	931428	931788	ribosomal protein L27 (rpL27) (Escherichia coli)	87.1	90.6	85
HI0953	1010494	1010261	ribosomal protein L28 (rpL28) (Escherichia coli)	85.7	94.8	77
HI0787	842654	842842	ribosomal protein L29 (rpL29) (Escherichia coli)	75.8	87.1	62
HI0779	838481	839104	ribosomal protein L3 (rpL3) (Escherichia coli)	85.2	92.3	209
HI0798	847813	847989	ribosomal protein L30 (rpL30) (Escherichia coli)	79.7	86.4	59
HI0760	821826	821617	ribosomal protein L31 (rpL31) (Escherichia coli)	71.4	85.7	70
HI0159	174441	174274	ribosomal protein L32 (rpL32) (Escherichia coli)	77.2	86.0	57
HI0952	1010246	1010079	ribosomal protein L33 (rpL33) (Escherichia coli)	81.5	90.7	54
HI1000	1063233	1063364	ribosomal protein L34 (rpL34) (Escherichia coli)	86.4	93.2	44
HI1322	1395096	1395269	ribosomal protein L35 (rpL35) (Escherichia coli)	75.0	90.6	32
HI0780	839123	839722	ribosomal protein L4 (rpL4) (Escherichia coli)	83.6	93.0	201
HI0792	845090	845626	ribosomal protein L5 (rpL5) (Escherichia coli)	90.5	96.1	179
HI0795	846391	846921	ribosomal protein L6 (rpL6) (Escherichia coli)	75.1	90.4	177
HI0643	681915	682283	ribosomal protein L7/L12 (rpL7/L12) (Escherichia coli)	82.0	91.8	121
HI0546	567619	567173	ribosomal protein L9 (rpL9) (Escherichia coli)	72.5	85.9	149
HI1223	1289629	1291274	ribosomal protein S1 (rpS1) (Escherichia coli)	79.3	88.7	557
HI0778	838108	838461	ribosomal protein S10 (rpS10) (Escherichia coli)	98.1	99.0	103

HI0802	850416	850802	ribosomal protein S11 (pS11) [Escherichia coli]		92.2	96.1	129
HI0801	850045	850397	ribosomal protein S13 (pS13) [Escherichia coli]		86.4	93.2	118
HI0793	845641	845943	ribosomal protein S14 (pS14) [Escherichia coli]		89.9	94.9	99
HI1331	1405806	1406072	ribosomal protein S15 (pS15) [Escherichia coli]		80.9	86.5	89
HI1473	1554091	1553825	ribosomal protein S15 (pS15) [Escherichia coli]		80.9	86.5	89
HI0205	218422	218177	ribosomal protein S16 (pS16) [Escherichia coli]		70.7	85.4	82
HI0788	842845	843099	ribosomal protein S17 (pS17) [Escherichia coli]		85.7	94.0	84
HI0547	567863	567639	ribosomal protein S18 (pS18) [Escherichia coli]		92.0	94.7	75
HI0783	840886	841158	ribosomal protein S19 (pS19) [Escherichia coli]		90.1	97.8	91
HI0915	967289	968041	ribosomal protein S2 (pS2) [Escherichia coli]		82.2	89.2	241
HI0533	553446	553658	ribosomal protein S21 (pS21) [Escherichia coli]		83.1	87.3	71
HI0785	841523	842227	ribosomal protein S3 (pS3) [Escherichia coli]		87.2	93.2	233
HI0803	850833	851450	ribosomal protein S4 (pS4) [Escherichia coli]		89.3	94.7	206
HI0797	847306	847803	ribosomal protein S5 (pS5) [Escherichia coli]		92.8	95.8	166
HI0549	568566	568192	ribosomal protein S6 (pS6) [Escherichia coli]		76.8	87.2	125
HI1537	1604087	1603182	ribosomal protein S6 modification protein (rimK) [Escherichia coli]		45.3	69.0	272
HI0582	599803	599336	ribosomal protein S7 (pS7) [Escherichia coli]		89.7	94.2	155
HI0794	845983	846372	ribosomal protein S8 (pS8) [Escherichia coli]		86.2	90.8	130
HI1446	1530328	1529939	ribosomal protein S9 (pS9) [Haemophilus somnus]		94.6	98.5	130
HI0010	11292	10828	ribosomal-protein-alanine acetyltransferase (rimI) [Escherichia coli]		55.9	73.1	144
HI0583	600334	599963	streptomycin resistance protein (strA) [Haemophilus influenzae]		100.0	100.0	124
	Amino acyl tRNA Synthetases, tRNA modification						
HI0816	865547	862926	alanyl-tRNA synthetase (alaS) [Escherichia coli]		68.2	82.6	873
HI1589	1648685	1650415	arginyl-tRNA synthetase (argS) [Escherichia coli]		71.2	83.5	577
HI1305	1382405	1380975	asparaginyl-tRNA synthetase (asnS) [Escherichia coli]		80.6	90.8	465
HI0319	348931	347168	aspartyl-tRNA synthetase (aspS) [Escherichia coli]		76.2	85.5	585
HI0078	85367	83991	cys-tRNA synthetase (cysS) [Escherichia coli]		75.7	87.0	461
HI0710	753356	754738	cysteinyl-tRNA (ser) selenium transferase (selA) [Escherichia coli]		58.8	75.8	454

HI1357	1431798	1433466	glutaminyl-tRNA synthetase (glnS) (Escherichia coli)	75.7	86.9	547
HI0276	308282	306843	glutamyl-tRNA synthetase (glx) (Escherichia coli)	72.4	84.3	464
HI0929	985024	984119	glycyl-tRNA synthetase alpha chain (glyQ) (Escherichia coli)	90.6	94.6	299
HI0926	983065	981002	glycyl-tRNA synthetase beta chain (glyS) (Escherichia coli)	69.7	81.9	689
HI0371	392076	393344	histidine-tRNA synthetase (hisS) (Escherichia coli)	66.8	79.1	421
HI0964	1021072	1018250	isoleucyl-tRNA ligase (ileS) (Escherichia coli)	66.0	78.5	934
HI0923	976547	979129	leucyl-tRNA synthetase (leuS) (Escherichia coli)	72.3	82.2	859
HI1214	1278435	1276930	lysyl-tRNA synthetase (lysU) (Escherichia coli)	70.2	84.3	505
HI0838	885271	886269	lysyl-tRNA synthetase analog (genX) (Escherichia coli)	62.7	78.5	331
HI0625	662613	663566	methionyl-tRNA formyltransferase (fmt) (Escherichia coli)	65.0	77.4	313
HI1279	1353301	1351256	methionyl-tRNA synthetase (metG) (Escherichia coli)	69.0	83.3	677
HI0396	416278	415697	peptidyl-tRNA hydrolase (pth) (Escherichia coli)	64.2	80.5	190
HI1314	1387690	1388676	phenylalanyl-tRNA synthetase beta-subunit (pheS) (Escherichia coli)	75.0	82.0	327
HI1315	1388713	1391097	phenylalanyl-tRNA synthetase beta-subunit (pheT) (Escherichia coli)	65.3	80.1	795
HI0731	781970	783684	prolyl-tRNA synthetase (proS) (Escherichia coli)	74.9	86.8	570
HI1650	1709685	1708879	pseudouridylyl synthase I (hisT) (Escherichia coli)	69.2	82.7	260
HI0246	273589	272501	queuosine biosynthesis protein (queA) (Escherichia coli)	72.5	85.7	346
HI0201	215333	216439	selenium metabolism protein (selD) (Escherichia coli)	66.1	80.6	330
HI0110	117234	118520	seryl-tRNA synthetase (serS) (Escherichia coli)	77.6	86.5	430
HI1370	1453876	1455804	threonyl-tRNA synthetase (thrS) (Escherichia coli)	77.9	86.1	642
HI0245	272154	271009	transfer RNA-guanine transglycosylase (tgt) (Escherichia coli)	81.3	91.5	374
HI0203	217564	216827	tRNA (guanine-N1)-methyltransferase (M1G-methyltransferase) (tmD) (Escherichia coli)	83.2	93.0	244
HI0850	894301	895389	tRNA (uracil-5-)-methyltransferase (imaA) (Escherichia coli)	64.6	80.4	362
HI0068	71519	72451	tRNA delta(2)-isopentenylpyrophosphate transferase (trpX) (Escherichia coli)	69.8	87.4	300

HI1612	1671420	1672667	tRNA nucleotidyltransferase (cca) (Escherichia coli)	58.4	73.4	404
HI0242	270097	269807	tRNA-guanine-transglycosylase (tgt) (Escherichia coli)	62.4	81.7	92
HI0639	678958	677957	tryptophanyl-tRNA synthetase (trpS) (Escherichia coli)	78.1	86.2	334
HI1616	1676533	1675331	tyrosyl tRNA synthetase (tyrS) (Thiobacillus ferrooxidans)	53.6	72.6	398
HI1396	1490259	1487398	valyl-tRNA synthetase (valS) (Escherichia coli)	70.8	83.3	951
	Nucleoproteins					
HI0187	200140	200544	DNA binding protein (probable) (Bacillus subtilis)	43.4	64.2	106
HI1496	1568461	1568685	DNA-binding protein (rdgB) (Erwinia carotovora)	42.4	60.6	67
HI1593	1655153	1655554	DNA-binding protein H-NS (hns) (Escherichia coli)	47.4	65.2	135
HI0432	453511	453104	DNA-binding protein HU-ALPHA (NS2) (HU-2) (Escherichia coli)	78.9	86.7	90
	Proteins - translation and modification					
HI0848	893035	893757	disulfide oxidoreductase (por) (Haemophilus influenzae)	100.0	100.0	205
HI0987	1042200	1041082	DNA processing chain A (dprA) (Escherichia coli)	44.8	60.2	358
HI0916	968177	969025	elongation factor EF-Ts (tsf) (Escherichia coli)	71.4	85.0	280
HI0580	597082	595901	elongation factor EF-Tu (duplicate) (tufB) (Escherichia coli)	92.6	95.9	394
HI0634	671167	672348	elongation factor EF-Tu (duplicate) (tufB) (Escherichia coli)	92.6	95.9	394
HI0581	599249	597150	elongation factor G (tusA) (Escherichia coli)	84.6	92.0	704
HI0330	355617	355054	elongation factor P (elp) (Escherichia coli)	75.0	85.6	188
HI0069	72460	75402	glutamate-ammonia-ligase adenylyltransferase (glnE) (Escherichia coli)	52.5	69.7	914
HI1321	1394551	1394954	initiation factor 3 (infC) (Escherichia coli)	82.8	94.8	134
HI0550	569019	568768	initiation factor IF-1 (infA) (Escherichia coli)	94.4	98.6	72
HI1287	1360021	1362507	initiation factor IF-2 (infB) (Escherichia coli)	70.9	84.5	842
HI1155	1218859	1220211	maturation of antibiotic MccB17 (pmbA) (Escherichia coli)	60.8	78.7	450
HI1728	1794724	1793921	methionine aminopeptidase (map) (Escherichia coli)	64.3	79.8	262
HI0430	450570	451100	oxido-reductase (dsbB) (Escherichia coli)	43.8	68.8	174
HI1215	1279684	1278589	peptid chain release factor 2 (prfB) (Salmonella typhimurium)	81.7	93.7	365

HI1741	1811636	1813216	peptide-chain-release factor 3 (prfC) [Escherichia coli]	86.0	93.4	527
HI0079	85470	85976	peptidyl-prolyl cis-trans isomerase B (ppiB) [Escherichia coli]	71.3	80.5	163
HI1567	1631427	1630345	polypeptide chain release factor 1 (prfA) [Salmonella typhimurium]	72.5	88.3	360
HI0624	662011	662517	polypeptide deformylase (lomy/methionine deformylase) (def) [Escherichia coli]	65.1	79.9	169
HI0810	857270	856716	ribosome releasing factor (lrr) [Escherichia coli]	68.1	84.9	185
HI0575	593158	592940	rotamase, peptidyl prolyl cis-trans isomerase (slyD) [Escherichia coli]	50.7	73.1	67
HI0701	745982	745413	rotamase, peptidyl prolyl cis-trans isomerase (slyD) [Escherichia coli]	68.3	79.4	187
HI1334	1408450	1408923	transcription elongation factor (greA) [Escherichia coli]	79.7	89.9	158
HI0711	754738	756593	translation factor (selB) [Escherichia coli]	44.0	64.7	606
HI1216	1279817	1280503	xprA protein (xprA) [Escherichia coli]	45.4	67.4	227
Degradation of proteins, peptides, glycopeptides						
HI0877	927500	928801	aminopeptidase A (pepA) [Rickettsia prowazekii]	39.6	57.9	313
HI1711	1775967	1777439	aminopeptidase a/i (pepA) [Escherichia coli]	57.3	77.5	497
HI1620	1682194	1679588	aminopeptidase N (pepN) [Escherichia coli]	60.9	75.6	864
HI0818	867554	866265	aminopeptidase P (pepP) [Escherichia coli]	54.6	73.6	435
HI0716	762461	763039	ATP-dependent clp protease proteolytic component (clpP) [Escherichia coli]	71.0	88.1	193
HI0717	763052	764284	ATP-dependent protease ATPase subunit (clpX) [Escherichia coli]	70.2	83.2	413
HI0861	906379	908946	ATP-dependent protease binding subunit (clpB) [Escherichia coli]	77.4	88.6	857
HI0421	440910	442289	collagenase activity collagenase (prc) [Porphyromonas gingivalis]	31.1	53.4	206
HI0151	166695	165811	HFLC protein (hflC) [Escherichia coli]	58.5	78.2	329
HI0248	274175	276400	IgA1 protease (iga1) [Haemophilus influenzae]	28.6	51.5	759
HI0992	1047674	1053118	IgA1 protease (iga1) [Haemophilus influenzae]	99.8	99.9	1702
HI0249	278527	276401	IgA1 protease (iga1) [Haemophilus influenzae]	45.2	62.5	791
HI1327	1402067	1403869	lon protease (lon) [Bacillus brevis]	24.2	46.6	714

HI0215	229004	231046	oligopeptidase A (prfC) (Escherichia coli)	72.0	84.8	678
HI0677	716670	718121	peptidase D (pepD) (Escherichia coli)	56.8	72.2	485
HI0589	608542	607865	peptidase E (pepE) (Escherichia coli)	41.4	60.0	214
HI1351	1423832	1425067	peptidase T (pepT) (Salmonella typhimurium)	53.3	71.4	398
HI1262	1336467	1335070	periplasmic serine protease Do and heat shock protein (htrA) (Escherichia coli)	55.8	73.9	469
HI1603	1664636	1663212	probable ATP-dependent protease (sms) (Escherichia coli)	80.0	92.2	460
HI0724	768169	768786	proline dipeptidase (pepQ) (Escherichia coli)	53.7	70.2	204
HI0137	151209	151901	protease (prtH) (Porphyromonas gingivalis)	52.6	64.9	57
HI1547	1613228	1611384	protease IV (sppA) (Escherichia coli)	43.7	64.0	607
HI0152	167927	166698	protease specific for phage lambda cII repressor (hlkK) (Escherichia coli)	55.8	72.6	396
HI1688	1751031	1752089	putative protease (sohB) (Escherichia coli)	53.3	74.5	348
HI0532	553214	552189	sialoglycoprotease (gcp) (Pasteurella haemolytica)	81.8	91.5	319
Transport/binding proteins						
Amino acids, peptides, amines						
HI1183	1247387	1246659	arginine transport ATP-binding protein artP (artP) (Escherichia coli)	65.8	83.1	242
HI1180	1245250	1244570	arginine transport system permease protein (artM) (Escherichia coli)	55.7	79.9	218
HI1181	1245915	1245253	arginine transport system permease protein (artQ) (Escherichia coli)	59.0	77.8	229
HI0254	284235	283786	biopolymer transport protein (exbB) (Haemophilus influenzae)	96.0	98.7	150
HI0253	283779	283339	biopolymer transport protein (exbD) (Escherichia coli)	28.8	55.1	118
HI1734	1801710	1800520	branched chain aa transport system II carrier protein (braB) (Pseudomonas aeruginosa)	28.4	49.8	279
HI0885	935516	934149	D-alanine permease (dagA) (Alteromonas haloplanktis)	43.2	65.5	527
HI1188	1251117	1250128	dipeptide transport ATP-binding protein (dppD) (Escherichia coli)	74.2	84.0	326
HI1187	1250122	1249142	dipeptide transport ATP-binding protein (dppF) (Escherichia coli)	76.4	87.1	325
HI1126	1189626	1188709	dipeptide transport system permease protein (dppB) (Escherichia coli)	34.1	60.7	337

HI1190	1253029	1252031	dipeptide transport system permease protein (dppB) (Escherichia coli)	61.1	79.2	337
HI1189	1252013	1251130	dipeptide transport system permease protein (dppC) (Escherichia coli)	63.8	83.3	287
HI1536	1601926	1603137	glutamate permease (gltS) (Escherichia coli)	53.9	73.0	391
HI1081	1146102	1145389	glutamine transport system permease protein (glnP) (Escherichia coli)	37.6	59.0	212
HI1082	1146859	1146089	glutamine-binding periplasmic protein (glnH) (Escherichia coli)	28.4	48.2	222
HI0410	429066	428263	leucine-specific transport protein (livG) (Escherichia coli)	28.1	55.2	250
HI0227	255068	256375	membrane-associated component, LIV-II transport system (bmQ) (Salmonella typhimurium)	32.9	60.4	425
HI0214	228528	226987	oligopeptide binding protein (oppA) (Escherichia coli)	31.7	53.5	473
HI1127	1191333	1189710	oligopeptide binding protein (oppA) (Escherichia coli)	52.6	69.0	527
HI1124	1187751	1186783	oligopeptide transport ATP-binding protein (oppD) (Salmonella typhimurium)	77.2	85.0	320
HI1123	1186783	1185788	oligopeptide transport ATP-binding protein (oppF) (Salmonella typhimurium)	71.5	83.9	329
HI1125	1188696	1187764	oligopeptide transport system permease protein (oppC)C (Salmonella typhimurium)	71.1	87.4	300
HI1644	1702355	1704049	peptide transport periplasmic protein (sapA) (Salmonella typhimurium)	39.3	63.8	504
HI1647	1705898	1706944	peptide transport system ATP-binding protein (sapD) (Salmonella typhimurium)	62.4	80.0	330
HI1646	1705007	1705891	dipeptide transport system permease protein (dppC) (Escherichia coli)	36.2	59.9	279
HI1645	1704052	1705014	peptide transport system permease protein (sapB) (Salmonella typhimurium)	34.4	63.8	319
HI1182	1246638	1245922	periplasmic arginine-binding protein (arlI) (Pasteurella haemolytica)	58.6	73.4	234
HI1157	1221270	1222589	proton glutamate symport protein (gltP) (Bacillus caldotenax)	26.6	53.6	395
HI0592	611920	610616	putrescine transport protein (potE) (Escherichia coli)	77.2	88.0	434
HI0291	324543	323308	serine transporter (sdaC) (Escherichia coli)	61.0	77.8	411

HI1350	1423563	1422421	spermidine/putrescine transport ATP-binding protein (potA) (Escherichia coli)	68.1	83.1	378
HI1349	1422434	1421577	spermidine/putrescine transport system permease protein (potB) (Escherichia coli)	61.5	83.6	275
HI1348	1421548	1420808	spermidine/putrescine transport system permease protein (potC) (Escherichia coli)	72.4	88.9	243
HI0500	514110	513175	spermidine/putrescine-binding periplasmic protein precursor (potD) (Escherichia coli)	59.2	75.2	309
HI1347	1420732	1419596	spermidine/putrescine-binding periplasmic protein precursor (potD) (Escherichia coli)	54.1	71.6	330
HI0289	320539	321792	tryptophan-specific permease (mtr) (Escherichia coli)	55.8	72.5	396
HI0479	497829	499028	tyrosine-specific transport protein (tyrP) (Escherichia coli)	46.1	68.2	401
HI0530	551559	550342	tyrosine-specific transport protein (tyrP) (Escherichia coli)	45.4	65.4	404
	Cations					
HI0255	284871	284407	bacterioferritin comigratory protein (bcp) (Escherichia coli)	62.3	79.9	154
HI1275	1347862	1348650	ferric enterobactin transport ATP-binding protein (fepC) (Escherichia coli)	29.4	51.3	238
HI1475	1555193	1554435	ferric enterobactin transport ATP-binding protein (fepC) (Escherichia coli)	33.2	54.8	220
HI1471	1549654	1551853	ferrichrome-iron receptor (fhuA) (Escherichia coli)	26.4	48.9	710
HI1388	1479930	1480475	ferritin like protein (rsgA) (Escherichia coli)	57.4	79.0	162
HI1389	1480494	1480988	ferritin like protein (rsgA) (Escherichia coli)	57.3	73.8	164
HI0363	385804	384887	iron(III) dicitrate transport ATP-binding protein FECE (Escherichia coli)	35.9	56.4	220
HI1274	1347324	1347861	iron(III) dicitrate transport system permease protein (fecD) (Escherichia coli)	36.0	64.0	255



HI1037	1099321	1100265	magnesium and cobalt transport protein (corA) [Escherichia coli]	70.3	84.8	316
HI0097	103798	104679	major ferric iron binding protein precursor (fbp) [Neisseria gonorrhoeae]	69.7	82.3	293
HI1051	1114308	1114635	mercuric transport protein (merT) [Pseudomonas aeruginosa]	25.0	55.2	99
HI1052	1114651	1114926	mercury scavenger protein (merP) [Pseudomonas fluorescens]	29.3	45.7	91
HI0294	327396	327193	mercury scavenger protein (merP) [Pseudomonas fluorescens]	32.8	67.2	67
HI1531	1594953	1594219	molybdate-binding periplasmic protein precursor (modB) (Azotobacter vinelandii)	21.7	43.0	245
HI0226	254880	253681	NA(+)/H(+) antiporter 1 (nhaA) [Escherichia coli]	52.6	74.6	380
HI0429	448992	450557	Na+/H+ antiporter (nhaB) [Escherichia coli]	70.6	87.5	501
HI1110	1171933	1170530	Na+/H+ antiporter (nhaC) [Bacillus firmus]	37.5	62.0	382
HI0098	104899	106317	periplasmic-binding-protein-dependent iron transport protein (sfuB) [Serratia marcescens]	38.1	59.5	457
HI1479	1558763	1558167	periplasmic-binding-protein-dependent iron transport protein (sfuC) [Serratia marcescens]	39.9	58.0	197
HI0913	964424	966276	potassium efflux system (kelC) [Escherichia coli]	40.9	65.7	594
HI0292	326934	324769	potassium/copper-transporting ATPase A (copA) [Enterococcus faecalis]	42.9	64.4	723
HI1355	1429787	1428276	sodium/proline symporter (proline permease) (pulP) [Escherichia coli]	62.8	79.1	489
HI0252	283326	282517	tonB protein (tonB) [Haemophilus influenzae]	96.2	98.5	261
HI0627	664922	666362	TRK system potassium uptake protein (trkA) [Escherichia coli]	65.8	83.4	458
			Carbohydrates, organic alcohols & acids			
HI0020	22097	20661	2-oxoglutarate/malate translocator (SODiT1) [Spinacia oleracea]	35.8	59.6	452
HI0824	872894	873940	D-galactose-binding periplasmic protein (mgB) [Escherichia coli]	67.6	81.2	329
HI1113	1176024	1174516	D-xylose transport ATP-binding protein (xylG) [Escherichia coli]	71.5	85.8	501
HI1114	1177073	1176078	D-xylose-binding periplasmic protein (rbsB) [Escherichia coli]	76.0	88.4	328
HI1718	1785024	1783300	enzyme I (ptsI) [Salmonella typhimurium]	70.2	84.3	574
HI0182	194818	193967	formate transporter (formate channel) [Escherichia coli]	53.2	73.4	263
HI0450	471781	470285	fructose-permease IIA/FPR component (fruB) [Escherichia coli]	51.5	68.3	374
HI0448	469337	467670	fructose-permease IIBC component (fruA) [Escherichia coli]	57.2	72.2	552

HI0614	643282	642851	fucose operon protein (fucU) [Escherichia coli]	66.3	80.0	94
HI0692	733673	734464	glpF protein (glpF) [Escherichia coli]	73.6	87.2	258
HI1019	1080518	1081194	glpF protein (glpF) [Escherichia coli]	30.6	54.6	208
HI1017	1078404	1079867	gluconate permease (gntP) [Bacillus subtilis]	29.1	56.4	442
HI1717	1783237	1782740	glucose phosphotransferase enzyme III-glc (crr) [Escherichia coli]	73.2	83.3	169
HI0688	729474	730914	glycerol-3-phosphatase transporter (glpT) [Escherichia coli]	64.5	78.9	445
HI0504	517869	519347	high affinity ribose transport protein (rbsA) [Escherichia coli]	71.1	85.4	494
HI0505	519363	520331	high affinity ribose transport protein (rbsC) [Escherichia coli]	68.0	86.5	303
HI0503	517436	517852	high affinity ribose transport protein (rbsD) [Escherichia coli]	59.0	78.4	139
HI0612	642139	640856	L-fucose permease (lucP) [Escherichia coli]	35.6	57.9	413
HI1221	1288578	1286983	L-lactate permease (lctP) [Escherichia coli]	30.2	53.9	532
HI1735	1802527	1801757	lactam utilization protein (lamB) [Emmericella nidulans]	41.3	60.3	130
HI0825	874009	875526	mgIA protein (mgIA) [Escherichia coli]	73.9	84.6	506
HI0826	875546	876553	mgIC protein (mgIC) [Escherichia coli]	79.2	90.2	336
HI0506	520354	521229	periplasmic ribose-binding protein (rbsB) [Escherichia coli]	73.9	86.6	291
HI1719	1785361	1785107	phosphohistidinoprotein-hexose phosphotransferase (ptsH) [Escherichia coli]	77.6	88.2	85
HI0830	878480	878773	potassium channel homolog (kch) [Escherichia coli]	67.7	80.2	96
HI0154	170140	168807	putative aspartate transport protein (dcuA) [Escherichia coli]	46.4	69.9	436
HI0748	803856	805175	putative aspartate transport protein (dcuA) [Escherichia coli]	42.6	70.1	435
HI1112	1174509	1173385	ribose transport permease protein (xylH) [Escherichia coli]	69.8	84.1	371
HI1696	1759373	1760743	sodium- and chloride-dependent GABA transporter (Homo sapiens)	29.3	52.6	471
HI0738	790926	789403	sodium-dependent noradrenaline transporter (Homo sapiens)	31.1	54.2	523
Nucleosides, purines & pyrimidines						
HI1089	1151815	1151024	ribonucleotide transport ATP-binding protein (mkt) [Mycobacterium leprae]	42.2	61.5	244
HI1230	1296319	1295078	uracil permease (uraA) [Escherichia coli]	37.2	61.6	400

	Anions						
HI1104	1164213	1165028	cysteine synthetase (cysZ) (Escherichia coli)		53.7	76.3	190
HI1697	1761825	1760773	hydrophilic membrane-bound protein (modC) (Escherichia coli)		55.9	74.5	263
HI1698	1762501	1761815	hydrophobic membrane-bound protein (modB) (Escherichia coli)		65.9	84.8	223
HI1384	1477430	1476585	integral membrane protein (pstA) (Escherichia coli)		59.6	77.6	272
HI0356	380045	380764	nitrate transporter ATPase component (nasD) (Klebsiella pneumoniae)		34.9	57.8	254
HI1383	1475710	1476584	peripheral membrane protein B (pstB) (Escherichia coli)		77.0	86.8	256
HI1385	1478379	1477435	peripheral membrane protein C (pstC) (Escherichia coli)		57.3	78.7	300
HI1386	1479246	1478473	periplasmic phosphate-binding protein (pstS) (Escherichia coli)		49.8	67.7	256
HI1387	1479247	1479929	periplasmic phosphate-binding protein (pstS) (Escherichia coli)		63.8	75.4	69
HI1610	1669474	1670733	phosphate permease(YBR296C) (Saccharomyces cerevisiae)		35.6	60.0	551
	Other						
HI0060	62564	60804	ATP dependent translocator homolog (msbA) (Haemophilus influenzae)		100.0	100.0	458
HI0623	653683	662010	ATP-binding protein (abc) (Escherichia coli)		74.0	86.5	200
HI1625	1686470	1686186	cystic fibrosis transmembrane conductance regulator (Bos taurus)		35.3	60.8	233
HI0855	899042	900688	heme-binding lipoprotein (dppA) (Haemophilus influenzae)		98.9	99.3	547
HI0266	295639	298353	heme-hemopexin-binding protein (hxuA) (Haemophilus influenzae)		82.1	89.5	928
HI1476	1556199	1555189	hemin permease (hemU) (Yersinia enterocolitica)		36.1	62.7	325
HI0264	291684	293852	hemin receptor precursor (hemR) (Yersinia enterocolitica)		28.5	45.9	678
HI1712	1779487	1777481	high-affinity choline transport protein (betT) (Escherichia coli)		34.7	61.6	653
HI0663	705327	703054	lactoferrin binding protein (lbpA) (Neisseria meningitidis)		30.2	47.9	763
HI0610	637954	639336	Na+/sulfate cotransporter (Rattus norvegicus)		34.4	57.8	562
HI0977	1032420	1033871	pantothenate permease (panF) (Escherichia coli)		60.2	77.9	478
HI0714	760739	757488	transferrin binding protein 1 precursor (tbp1) (Neisseria meningitidis)		29.9	48.6	894
HI0996	1059604	1056869	transferrin binding protein 1 precursor (tbp1) (Neisseria meningitidis)		51.2	69.5	885

HI1220	1286725	1283987	transferrin binding protein 1 precursor (tbp1) (Neisseria meningitidis)	28.4	46.8	902
HI0997	1061509	1059635	transferrin binding protein 2 precursor (tbp2) (Neisseria meningitidis)	39.9	54.7	692
HI0975	1029676	1030542	transferrin-binding protein (tfbA) (Actinobacillus pleuropneumoniae)	28.9	48.0	578
HI1571	1633105	1633993	transferrin-binding protein 1 (tbp1) (Neisseria meningitidis)	41.3	59.5	727
HI0637	676956	674098	transferrin-binding protein 1 (tbp2) (Neisseria gonorrhoeae)	31.6	51.7	828
HI0665	706622	708309	transport ATP-binding protein (cydD) (Escherichia coli)	26.4	54.0	561
HI1160	1226897	1225140	transport ATP-binding protein (cydD) (Escherichia coli)	50.7	73.5	588
Cellular processes						
Chaperones						
HI0544	565037	565324	chaperonin (groES) (mopB) (Escherichia coli)	87.5	94.8	96
HI0545	565350	566993	heat shock protein (groEL) (mopA) (Haemophilus ducreyi)	89.8	94.9	547
HI1241	1310497	1311678	heat shock protein (dnaJ) (Escherichia coli)	68.0	82.5	376
HI0104	111572	109680	heat shock protein C62.5 (hlpG) (Escherichia coli)	75.4	88.3	621
HI0375	396463	394607	hsc66 protein (hsc66) (Escherichia coli)	69.2	82.0	616
HI1240	1308539	1310443	hsp70 protein (dnaK) (Escherichia coli)	78.5	88.2	638
Cell division						
HI0771	831200	831853	cell division ATP-binding protein (ftsE) (Escherichia coli)	64.1	78.3	216
HI1211	1275245	1274358	cell division inhibitor (sulA) (Vibrio cholerae)	33.9	55.7	116
HI1145	1210058	1211332	cell division protein (ftsA) (Escherichia coli)	52.8	74.2	420
HI1338	1410017	1412129	cell division protein (ftsH) (Escherichia coli)	75.2	87.8	624
HI1470	1549516	1548374	cell division protein (ftsH) (Escherichia coli)	77.8	88.3	369
HI1337	1409390	1410016	cell division protein (ftsJ) (Escherichia coli)	81.7	90.4	208
HI1134	1196901	1197221	cell division protein (ftsL) (Escherichia coli)	36.6	60.4	101
HI1144	1209275	1210036	cell division protein (ftsQ) (Escherichia coli)	40.6	58.5	231
HI1140	1204467	1205648	cell division protein (ftsW) (Escherichia coli)	52.3	74.9	374

HI0770	829937	831178	cell division protein (ftsY) (Escherichia coli)	66.0	81.1	497
HI1146	1211419	1212681	cell division protein (ftsZ) (Escherichia coli)	67.2	83.1	306
HI1377	1465224	1469760	cell division protein (mukB) (Escherichia coli)	61.4	77.3	1455
HI1356	1429903	1431375	cytoplasmic axial filament protein (cafA) (Escherichia coli)	71.0	86.3	488
HI0772	831866	832795	ftsX protein (ftsX) (Escherichia coli)	43.5	69.9	292
HI1067	1128511	1129221	mukB suppressor protein (smbA) (Escherichia coli)	77.4	90.2	235
HI1135	1197237	1199067	penicillin-binding protein 3 (ftsI) (Escherichia coli)	52.8	70.7	564
	Protein, peptide secretion					
HI0016	17278	15485	GTP-binding membrane protein (lepA) (Escherichia coli)	85.6	91.0	597
HI1472	1551915	1553681	colicin V secretion ATP-binding protein (cvaB) (Escherichia coli)	29.9	56.0	373
HI1008	1070885	1071397	lipoprotein signal peptidase (lspA) (Escherichia coli)	51.3	71.5	158
HI1648	1706947	1707753	peptide transport system ATP-binding protein SAPF (sapF) (Escherichia coli)	49.6	70.8	264
HI0718	764525	764842	preprotein translocase (secE) (Escherichia coli)	40.6	62.3	106
HI0800	848438	849760	preprotein translocase SECY subunit (secY) (Escherichia coli)	74.7	86.9	443
HI0241	269734	267887	protein-export membrane protein (secD) (Escherichia coli)	59.6	77.3	615
HI0240	267876	266902	protein-export membrane protein (secF) (Escherichia coli)	48.0	73.0	302
HI0447	466800	467135	protein-export membrane protein (secG) (Escherichia coli)	58.9	81.3	110
HI0745	801965	801459	protein-export protein (secB) (Escherichia coli)	56.2	80.8	145
HI0911	961135	963837	secA protein (secA) (Escherichia coli)	68.0	81.7	896
HI0015	15473	14427	signal peptidase I (lepB) (Escherichia coli)	46.3	65.1	319
HI0106	114073	112688	signal recognition particle protein (54 homolog) (flh) (Escherichia coli)	79.9	90.9	452
HI0715	761040	762335	trigger factor (tig) (Escherichia coli)	64.4	80.3	432
HI0298	330445	329756	type 4 prepilin-like protein specific leader peptidase (hopD) (Escherichia coli)	27.2	49.0	208
HI0299	331661	330445	xcpS protein (xcpS) (Pseudomonas putida)	29.2	56.7	396

[illegible]

[illegible]

HI1522	1586368	1587105	P protein (Bacteriophage mu)	39.5	67.3	220
HI1416	1505940	1505428	terminase subunit 1 (Bacteriophage SF6)	32.3	52.3	128
HI1483	1560600	1562660	transposase A (muA) (Bacteriophage mu)	40.6	60.1	596
			Transposon-related functions			
HI1106	1166078	1166803	insertion sequence IS1016(V-4) hypothetical protein (GB:X58176.2) (Haemophilus influenzae)	43.6	66.7	39
HI1020	1081916	1081346	IS1016-V6 protein (IS1016-V6) (Haemophilus influenzae)	91.7	93.8	191
HI1332	1406795	1406150	IS1016-V6 protein (IS1016-V6) (Haemophilus influenzae)	54.7	74.7	170
HI1583	1645515	1645991	IS1016-V6 protein (IS1016-V6) (Haemophilus influenzae)	45.4	61.2	153
			Drug/analog sensitivity			
HI0897	947919	951014	acriflavine resistance protein (acrB) (Escherichia coli)	32.7	55.0	1027
HI0302	333614	334165	ampD signalling protein (ampD) (Escherichia coli)	56.1	75.1	172
HI1245	1315822	1314629	bicyclomycin resistance protein (bcr) (Escherichia coli)	42.6	68.7	383
HI1629	1688581	1689111	mercury resistance regulatory protein (merR2) (Thiobacillus ferrooxidans)	37.7	57.5	105
HI0650	692523	691900	modulator of drug activity (mda66) (Escherichia coli)	58.1	75.4	191
HI0899	953570	952041	multidrug resistance protein (emrB) (Escherichia coli)	67.7	84.8	499
HI0900	954752	953583	multidrug resistance protein (ermA) (Escherichia coli)	46.5	66.3	389
HI0036	37441	39472	multidrug resistance protein (mdl) (Escherichia coli)	29.0	51.2	1094
HI1467	1543471	1544832	nodulation protein T (nodT) (Rhizobium leguminosarum)	20.1	46.3	390
HI0551	569189	570049	rRNA (adenosine-N6,N6'-dimethyltransferase (ksgA) (Escherichia coli)	69.3	81.5	269
HI0513	527345	526362	tellurite resistance protein (tehA) (Escherichia coli)	38.9	62.0	317
HI1278	1351140	1350283	tellurite resistance protein (tehB) (Escherichia coli)	55.2	70.6	194
			Radiation sensitivity			
HI0954	1011412	1010711	radC protein (radC) (Escherichia coli)	49.8	71.7	219



	Adaptations, atypical conditions					
HI1532	1596570	1595143	autotrophic growth protein (aut) (Alcaligenes eutrophus)	45.0	60.9	154
HI0722	766921	767769	heat shock protein (hspX) (Escherichia coli)	66.3	82.1	288
HI1533	1596655	1597599	heat shock protein B (hspB) (Escherichia coli)	55.9	71.2	304
HI0947	1003887	1004906	htrA-like protein (htrH) (Escherichia coli)	55.2	72.6	262
HI0903	956705	957292	invasion protein (invA) (Bartonella bacilliformis)	39.5	60.5	167
HI1550	1615090	1614485	NAD(P)H:menadiene oxidoreductase (Mus musculus)	35.9	54.9	200
HI0460	479443	478505	survival protein (surA) (Escherichia coli)	33.0	58.5	424
HI0817	866160	865738	uspA protein (uspA) (Escherichia coli)	68.6	87.1	140
HI0323	350541	350774	virulence plasmid protein (vagC) (Salmonella dublin)	35.9	57.8	62
HI1254	1326770	1327090	virulence associated protein A (vapA) (Dichelobacter nodosus)	40.8	57.7	71
HI0324	350774	351175	virulence associated protein C (vapC) (Dichelobacter nodosus)	35.4	56.9	128
HI0949	1007984	1007589	virulence associated protein C (vapC) (Dichelobacter nodosus)	40.9	60.6	131
HI0452	472751	472479	virulence associated protein D (vapD) (Dichelobacter nodosus)	40.7	67.0	91
HI1310	1385051	1385680	virulence plasmid protein (mlgA) (Shewanella colwelliana)	23.8	56.3	124
	Undetermined					
HI1164	1230321	1229908	15 kDa protein (P15) (Escherichia coli)	49.3	68.4	136
HI0085	89585	88593	2-hydroxyaciddehydrogenases homolog (ddh) (Zymomonas mobilis)	51.5	72.8	324
HI0462	480185	480973	beta-lactamase regulatory homolog (mazG) (Escherichia coli)	48.3	72.6	257
HI1676	1738223	1737753	conjugative transfer co-repressor (finO) (Escherichia coli)	32.5	51.9	76
HI0309	340039	340851	delta-1-pyrroline-5-carboxylate reductase (proC) (Pseudomonas aeruginosa)	44.0	60.1	267
HI1555	1620490	1619810	devA protein (devA) (Anabaena sp.)	42.7	66.4	219
HI0558	576002	575514	devB protein (devB) (Anabaena sp.)	32.7	51.5	166
HI1342	1415087	1415473	embryonic abundant protein, group 3 (Triticum aestivum)	33.3	50.0	102
HI0939	996457	995658	extragenic suppressor (suhB) (Escherichia coli)	64.7	80.2	258

HI0370	390960	392063	GCPE protein (protein E) (gpcE) (Escherichia coli)	88.2	93.9	362
HI0095	102616	101864	GerC2 protein (gerC2) (Bacillus subtilis)	32.9	55.2	191
HI0669	712892	711894	glpX protein (glpX) (Escherichia coli)	69.2	83.4	325
HI1015	1076616	1077389	glyoxylate-induced protein (Escherichia coli)	39.1	57.8	258
HI0499	511702	513099	hslU protein (hslU) (Escherichia coli)	80.4	90.1	443
HI0498	511230	511754	hslV protein (hslV) (Escherichia coli)	79.8	89.0	172
HI1120	1184041	1182516	ilv-related protein (Escherichia coli)	59.7	77.0	504
HI0287	319073	317784	isochorismate synthase (entC) (Bacillus subtilis)	31.5	48.9	311
HI1624	1686217	1685567	membrane associated ATPase (cbfO) (Propionibacterium freudenreichii)	33.7	52.7	184
HI0463	481901	481029	membrane protein (lapB) (Pasteurella haemolytica)	34.2	56.0	221
HI1122	1184867	1185742	membrane protein (lapB) (Pasteurella haemolytica)	63.1	80.2	216
HI0590	608642	609874	N-carbamyl-L-amino acid amidohydrolase (Bacillus stearothermophilus)	35.9	59.2	406
HI0380	399796	398579	nitrogen fixation protein (nifS) (Anabaena sp.)	48.2	67.0	379
HI1298	1375045	1373735	nitrogen fixation protein (nifS) (Mycobacterium leprae)	33.4	56.2	402
HI1346	1418236	1417523	nitrogen fixation protein (nifS) (Mycobacterium leprae)	38.8	58.5	186
HI0379	398591	398139	nitrogen fixation protein (nifU) (Klebsiella pneumoniae)	50.8	74.2	122
HI0167	180354	181586	nitrogen fixation protein (nifE) (Rhodobacter capsulatus)	30.1	47.9	292
HI1692	1756087	1757160	nitrogen fixation protein (nifE) (Rhodobacter capsulatus)	32.7	59.5	290

HI0129	143015	144800	nitrogenase C (nifC) (Clostridium pasteurianum)	27.1	52.6	248
HI1480	1559124	1558768	nitrogenase C (nifC) (Clostridium pasteurianum)	40.9	60.2	92
HI0359	381523	382464	nmt1 protein (nmt1) (Aspergillus parasiticus)	25.6	54.7	289
HI1299	1375415	1374882	partitioning system protein (parB) (Plasmid RP4)	43.6	67.7	141
HI0224	252941	252168	rarD protein (rarD) (Escherichia coli)	26.5	53.0	230
HI0682	721733	720840	rarD protein (rarD) (Escherichia coli)	27.1	55.0	289
HI0918	970839	970249	skp protein (skp) (Pasteurella multocida)	55.5	76.4	191
HI0983	1038375	1037893	small protein (smpB) (Escherichia coli)	78.8	91.3	160
HI1598	1661468	1659882	spoIIIE protein (spoIIIE) (Coxiella burnetii)	56.1	74.5	504
HI0898	951407	952018	suppressor protein (msgA) (Escherichia coli)	30.2	56.1	254
HI1080	1145382	1144612	surfactin (sfpo) (Bacillus subtilis)	58.2	77.9	246
HI0753	811790	811296	toxR regulon (tagD) (Vibrio cholerae)	45.7	64.0	164
HI1412	1502860	1501311	traN protein (traN) (Plasmid RP4)	40.2	61.5	233
HI0666	708305	709960	transport ATP-binding protein (cydC) (Escherichia coli)	26.3	51.7	536
HI1159	1225137	1223410	transport ATP-binding protein (cydC) (Escherichia coli)	48.5	70.1	568
HI1562	1627239	1626295	vanH protein (vanH) (Transposon Tn1546)	39.7	57.1	251
HI0632	668489	669433	mucoid status locus protein (mucB) (Pseudomonas aeruginosa)	25.4	51.8	309
HI0172	183553	184785	phenolhydroxylase (ORF6) (Acinetobacter calcoaceticus)	33.0	56.9	313
HI1390	1481177	1481266	plasma protease C1 inhibitor (Homo sapiens)	75.0	79.2	23

KNOWN.before

HI0060	ATP dependent translocator homolog (msbA)
HI0140	outer membrane protein P2 (ompP2)
HI0251	single-stranded DNA binding protein (ssb)
HI0252	tonB protein (tonB)
HI0266	heme-hemopexin-binding protein (hxaA)
HI0351	adenylate kinase (ATP-AMP transphosphorylase) (adk)
HI0352	hypothetical protein (SP:P24326)
HI0353	udp-glucose 4-epimerase (galactowaldenase) (galE)
HI0354	hypothetical protein (SP:P24324)
HI0383	PC protein (15kd peptidoglycan-associated outer membrane lipoprotein) (pal)
HI0403	outer membrane protein P1 (ompP1)
HI0435	transformation gene cluster hypothetical protein (GB:M62809_10) (com)
HI0436	com101A protein (comF)
HI0437	transformation gene cluster hypothetical protein (GB:M62809_7) (com)
HI0438	transformation gene cluster hypothetical protein (GB:M62809_6) (com)
HI0439	transformation gene cluster hypothetical protein (GB:M62809_5) (com)
HI0440	transformation gene cluster hypothetical protein (GB:M62809_4) (com)
HI0441	transformation gene cluster hypothetical protein (GB:M62809_3) (com)
HI0442	transformation gene cluster hypothetical protein (GB:M62809_2) (com)
HI0443	transformation gene cluster hypothetical protein (GB:M62809_1) (com)
HI0514	HincII endonuclease (HincII)
HI0515	modification methylase HincII (hincIIM)

Table 1(b)

HI0552	lipooligosaccharide biosynthesis protein
HI0583	streptomycin resistance protein (strA)
HI0602	recombinase (recA)
HI0603	tfoX protein (tfoX)
HI0606	adenylate cyclase (cyaA)
HI0622	28 kDa membrane protein (hlpA)
HI0691	protein D (hpd)
HI0695	lipoprotein (hel)
HI0820	aldose 1-epimerase precursor (mutarotase) (mro)
HI0821	galactokinase (galk)
HI0822	galactose-1-phosphate uridylyltransferase (galT)
HI0823	galactose operon repressor (galS)
HI0847	hypothetical protein (GB:M94205_1)
HI0848	disulfide oxidoreductase (por)
HI0855	heme-binding lipoprotein (dppA)
HI0919	protective surface antigen D15
HI0930	KW20 catalase (hktE)
HI0959	cyclic AMP receptor protein (crp)
HI1090	superoxide dismutase (sodA)
HI1167	outer membrane protein P5 (ompA)
HI1191	DNA helicase II (uvrD)
HI1397	HindIII modification methyltransferase (hindIIIM)
HI1398	HindIII restriction endonuclease (hindIIIR)
HI1402	DNA polymerase III, chi subunit (holC)

KNOWN.before

HI1545	lic-1 operon protein (licC)
HI1546	lic-1 operon protein (licD)
HI1585	15 kd peptidoglycan-associated lipoprotein (lpp)
HI1594	formyltetrahydrofolate hydrolase (purU)
HI1595	enolpyruvylshikimatephosphatesynthase (aroA)
HI1699	lsg locus hypothetical protein (GB:M94855_8)
HI1700	lsg locus hypothetical protein (GB:M94855_7)
HI1701	lsg locus hypothetical protein (GB:M94855_6)
HI1702	lsg locus hypothetical protein (GB:M94855_5)
HI1703	lsg locus hypothetical protein (GB:M94855_4)
HI1704	lsg locus hypothetical protein (GB:M94855_3)
HI1705	lsg locus hypothetical protein (GB:M94855_2)
HI1706	lsg locus hypothetical protein (GB:M94855_1)

Table 2

## UNKNOWN

HI0003	3249	2464	HI0147	160966	161952	HI0293	327115	326912
HI0004	3729	3268	HI0148	161966	163864	HI0295	327473	327856
HI0012	11778	12767	HI0149	164031	165167	HI0301	333498	333052
HI0017	17829	17449	HI0150	165574	165762	HI0305	337302	338036
HI0019	20239	18819	HI0153	168744	168040	HI0306	338036	338593
HI0021	23349	22102	HI0160	174988	174467	HI0307	338596	339012
HI0028	29582	29307	HI0163	178311	177715	HI0308	339973	339068
HI0033	35298	34834	HI0165	179007	180080	HI0310	340854	342017
HI0034	35660	35355	HI0166	180130	180348	HI0312	343117	343401
HI0035	37440	35788	HI0168	181582	182313	HI0313	343271	343092
HI0040	43059	42286	HI0169	182316	182567	HI0317	346507	345770
HI0042	44594	43923	HI0170	182570	182938	HI0318	347143	346670
HI0043	45658	44597	HI0171	182945	183537	HI0320	349150	349665
HI0044	46380	45721	HI0173	184932	185969	HI0321	349721	350002
HI0045	47261	46710	HI0174	185975	186232	HI0322	349998	350444
HI0046	47328	47687	HI0175	186247	187500	HI0325	351245	351649
HI0050	51426	50224	HI0176	188281	187550	HI0327	352729	354078
HI0051	51998	51504	HI0177	189257	188286	HI0328	354114	354374
HI0052	53023	52040	HI0178	189365	190150	HI0329	354653	354697
HI0053	54078	53053	HI0179	190715	190236	HI0331	355655	356668
HI0056	56966	56256	HI0183	195295	196233	HI0335	359242	360555
HI0059	60728	59733	HI0184	196413	197855	HI0338	363320	363910
HI0065	67839	68312	HI0185	198872	198048	HI0340	364253	365296
HI0072	78167	77313	HI0188	200705	201555	HI0342	367615	368352
HI0073	79220	78879	HI0189	201568	202335	HI0343	368440	368781
HI0074	79653	79216	HI0196	208646	208611	HI0344	368990	369516
HI0077	83046	83909	HI0199	213460	214224	HI0345	369512	369790
HI0080	85983	86411	HI0204	218138	217605	HI0346	369815	372311
HI0081	86556	87341	HI0206	218715	219485	HI0347	372369	373205
HI0082	87601	87864	HI0211	225095	225199	HI0348	373208	374068

HI0083	87882	88094	HI0218	234170	234697	HI0349	374068	374517
HI0090	96604	97314	HI0220	238722	238084	HI0352	377303	376029
HI0091	98493	97360	HI0228	256953	256489	HI0354	379329	378637
HI0092	99761	98505	HI0229	257403	257032	HI0355	379330	380044
HI0093	100989	99886	HI0231	259913	260854	HI0357	380765	381167
HI0094	101511	101194	HI0233	262997	264382	HI0358	381227	381171
HI0096	102950	103522	HI0234	264390	264539	HI0361	384039	383227
HI0100	107807	107415	HI0235	264822	264679	HI0365	386932	387009
HI0101	108091	107654	HI0236	265239	265033	HI0366	387928	387053
HI0103	109598	109257	HI0238	265736	266389	HI0367	388154	389323
HI0105	111789	112625	HI0239	266350	266781	HI0368	389428	389964
HI0107	114405	115612	HI0243	270426	270208	HI0369	390039	390947
HI0108	115744	116634	HI0244	270941	270426	HI0372	393364	393975
HI0109	117067	116729	HI0247	274159	273716	HI0373	394223	394032
HI0112	119485	119847	HI0257	285979	286623	HI0376	397168	396485
HI0114	122424	122311	HI0258	286796	286879	HI0377	397743	397222
HI0115	128606	130242	HI0259	286880	288054	HI0378	398079	397759
HI0116	130860	130246	HI0260	288240	288058	HI0381	400309	399860
HI0117	131552	131800	HI0261	288839	288180	HI0382	401087	400365
HI0120	134883	134380	HI0262	289503	288919	HI0388	406077	405670
HI0121	136357	134999	HI0267	298808	298450	HI0390	408337	409044
HI0125	140096	141409	HI0268	298891	299487	HI0391	409072	409620
HI0126	142556	141573	HI0272	304213	303284	HI0393	413144	412599
HI0127	142955	143011	HI0273	305079	304216	HI0394	414371	413637
HI0128	142718	142584	HI0277	309032	310684	HI0395	415645	414557
HI0130	145160	144804	HI0278	311516	310710	HI0397	416445	416750
HI0131	145840	145136	HI0279	311998	311516	HI0398	416756	417967
HI0134	147247	148419	HI0280	312417	312004	HI0400	419468	420118
HI0135	148422	149609	HI0281	312664	312371	HI0402	421340	421056
HI0136	151208	149695	HI0283	315199	313886	HI0406	425499	424210
HI0144	159021	158125	HI0284	315200	316061	HI0407	426365	425502
HI0146	160156	159932	HI0286	318836	319252	HI0414	433167	432202



HI0417	437163	437957	HI0600	620322	619999	HI0809	856603	855413
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HI0419	438773	439450	HI0626	663569	664921	HI0819	868114	867569
HI0420	439398	440738	HI0628	666387	666770	HI0827	876702	877433
HI0422	442434	442730	HI0629	666863	667117	HI0828	877442	877996
HI0423	443077	442916	HI0635	672600	672893	HI0829	877989	878460
HI0425	444797	445516	HI0636	672899	673879	HI0833	881059	881640
HI0426	446607	445555	HI0638	677932	677645	HI0839	887221	886541
HI0433	454103	453516	HI0640	679087	679701	HI0840	887844	887278
HI0434	454932	454142	HI0649	691619	690906	HI0841	888779	887757
HI0444	463691	464053	HI0652	694996	694787	HI0842	888896	889111
HI0451	472389	471856	HI0655	696806	697567	HI0843	889116	890870
HI0453	472951	472763	HI0658	699494	698946	HI0844	891071	891898
HI0454	474321	473026	HI0660	701972	700059	HI0845	891925	892059
HI0455	474896	474375	HI0661	702429	702136	HI0847	892866	893129
HI0456	475705	474926	HI0662	702781	702425	HI0849	893822	894164
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HI0466	485905	486561	HI0667	711078	710050	HI0852	896141	896572
HI0468	488712	487873	HI0668	711395	711078	HI0853	896977	897510
HI0469	489585	488725	HI0670	713054	713269	HI0854	897510	898898
HI0471	491037	492317	HI0672	713806	714236	HI0856	900867	901625
HI0478	497647	497796	HI0673	715017	714544	HI0857	902112	901768
HI0489	507333	506959	HI0674	715691	714544	HI0859	905068	905367
HI0490	507449	508048	HI0675	715969	715694	HI0860	905688	906248
HI0491	508051	508521	HI0679	719498	719061	HI0862	909726	908989
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HI0494	509815	509856	HI0696	737789	738508	HI0866	915792	913945
HI0495	509856	510253	HI0698	743511	739619	HI0868	918419	918538
HI0496	510797	510306	HI0699	744964	743524	HI0871	920692	921246
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HI0509	523382	523930	HI0703	746632	747648	HI0876	927351	926155

HI0510	524561	524076	HI0704	747649	748418	HI0880	931427	930509
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HI0512	525587	526303	HI0708	749180	749148	HI0884	933350	934084
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HI0528	549859	549044	HI0727	776875	777312	HI0908	959765	960283
HI0554	571956	572576	HI0732	786122	783778	HI0909	960628	960317
HI0556	575147	574608	HI0733	786625	786245	HI0910	960708	961007
HI0557	575547	575211	HI0734	786731	786582	HI0914	966380	967141
HI0559	576210	576091	HI0735	787647	786715	HI0920	974685	973357
HI0562	578540	580381	HI0737	788457	789167	HI0922	976298	975582
HI0563	581038	580382	HI0742	799454	800908	HI0927	983767	983405
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HI0588	607795	607361	HI0769	829882	829304	HI0942	997886	998566
HI0591	610092	610508	HI0774	835432	834092	HI0943	998544	998846
HI0594	614632	614441	HI0775	836100	835432	HI0945	1002315	1002762
HI0595	616566	616775	HI0777	836970	837914	HI0950	1008217	1007987
HI0596	616702	615176	HI0789	843493	844095	HI0957	1013246	1013899
HI0599	619155	619970	HI0808	854572	855375	HI0958	1013924	1014091

HI0960	1016378	1015203	HI1091	1153141	1153776	HI1258	1330618	1330839
HI0961	1017426	1016374	HI1092	1153784	1154446	HI1259	1330839	1331300
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HI0965	1022039	1021104	HI1095	1155489	1156007	HI1268	1346269	1345733
HI0966	1023606	1022077	HI1096	1156007	1157950	HI1269	1346756	1346836
HI0967	1023993	1024175	HI1097	1158092	1158634	HI1270	1346624	1346241
HI0968	1024843	1024944	HI1098	1158637	1160013	HI1271	1346849	1347025
HI0969	1024817	1024254	HI1099	1160451	1160492	HI1272	1347022	1347135
HI0976	1030609	1031712	HI1100	1160501	1160632	HI1273	1347135	1347323
HI0978	1033994	1034863	HI1101	1160637	1160942	HI1276	1348650	1349453
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HI0981	1036523	1037512	HI1107	1166804	1168024	HI1284	1356655	1357185
HI0986	1041067	1040252	HI1121	1184774	1184115	HI1285	1358080	1358502
HI0988	1042709	1044301	HI1128	1191629	1192577	HI1289	1367227	1365851
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HI0998	1061607	1062044	HI1131	1195069	1195242	HI1292	1369450	1370385
HI0999	1062363	1063049	HI1132	1195447	1195899	HI1294	1372453	1371617
HI1002	1063710	1063967	HI1133	1195933	1196895	HI1295	1373365	1372583
HI1003	1063970	1065592	HI1149	1215838	1214972	HI1296	1373601	1373359
HI1005	1067299	1067478	HI1150	1216338	1215847	HI1297	1373735	1373532
HI1006	1067384	1069165	HI1151	1217066	1216344	HI1300	1375530	1375949
HI1007	1069256	1070812	HI1152	1217588	1217073	HI1301	1375971	1376663
HI1009	1071385	1072338	HI1153	1218198	1217572	HI1303	1378236	1380176
HI1012	1073835	1074737	HI1154	1218770	1218237	HI1304	1380896	1380210
HI1013	1074743	1075981	HI1156	1220425	1220961	HI1309	1384563	1385051
HI1016	1077448	1078392	HI1158	1223159	1222695	HI1312	1386755	1386510
HI1018	1079890	1080315	HI1165	1231243	1230773	HI1313	1386780	1387538
HI1021	1082175	1083170	HI1168	1235872	1236231	HI1317	1391445	1391927
HI1022	1083178	1084791	HI1171	1238778	1239119	HI1318	1392096	1392410
HI1023	1084736	1085422	HI1172	1239729	1239166	HI1319	1392802	1393383
HI1026	1089466	1088792	HI1176	1242916	1243383	HI1320	1393468	1394280

HI1028	1091065	1090208	HI1178	1244125	1244051	HI1326	1401970	1401527
HI1029	1091066	1092597	HI1179	1244360	1244142	HI1329	1404808	1405533
HI1030	1093581	1092598	HI1184	1248098	1247517	HI1330	1405533	1405667
HI1031	1094889	1093615	HI1185	1248305	1248859	HI1335	1409063	1408968
HI1032	1095371	1094889	HI1186	1248934	1249107	HI1336	1409263	1408968
HI1033	1096441	1095446	HI1193	1256974	1256552	HI1340	1412995	1414329
HI1034	1096617	1097420	HI1194	1257654	1257067	HI1341	1414391	1414882
HI1036	1098535	1099023	HI1195	1257810	1257950	HI1343	1416879	1415557
HI1038	1100259	1100810	HI1198	1260250	1261479	HI1344	1417617	1417009
HI1039	1101878	1100997	HI1201	1263689	1264309	HI1345	1418133	1419509
HI1040	1102257	1103456	HI1202	1264360	1265430	HI1352	1426116	1425637
HI1041	1103535	1103386	HI1205	1267550	1268050	HI1354	1428276	1427314
HI1045	1108332	1107835	HI1206	1270263	1268131	HI1358	1433535	1433996
HI1046	1108943	1108335	HI1208	1271751	1272191	HI1367	1450229	1449366
HI1050	1113198	1114304	HI1218	1282515	1283219	HI1369	1453591	1453010
HI1055	1117984	1118322	HI1219	1283219	1283904	HI1371	1458706	1455929
HI1056	1119807	1118428	HI1225	1291759	1292049	HI1372	1461329	1458813
HI1057	1121239	1119698	HI1226	1292052	1293239	HI1378	1469827	1470732
HI1058	1123210	1123287	HI1237	1306218	1306673	HI1379	1470738	1471610
HI1060	1123449	1122868	HI1238	1307299	1306835	HI1391	1481365	1481808
HI1065	1127036	1126827	HI1239	1308273	1307173	HI1394	1484556	1485554
HI1066	1128454	1127000	HI1243	1313696	1313037	HI1399	1492391	1492023
HI1072	1135049	1133604	HI1244	1313794	1314591	HI1400	1493035	1492616
HI1073	1135234	1134995	HI1246	1316522	1315827	HI1401	1493171	1493004
HI1074	1137513	1135267	HI1247	1317233	1316616	HI1404	1495447	1496052
HI1075	1137884	1137513	HI1249	1319911	1321851	HI1405	1496978	1496157
HI1076	1138337	1137888	HI1251	1325506	1324541	HI1407	1498433	1498230
HI1084	1148702	1148448	HI1252	1326129	1325512	HI1408	1499014	1498469
HI1085	1149040	1148726	HI1253	1326454	1326756	HI1409	1499166	1499050
HI1086	1149695	1149054	HI1255	1327256	1328923	HI1410	1500612	1499515
HI1087	1150228	1149728	HI1256	1328946	1329326	HI1411	1501029	1500676
HI1088	1151024	1150242	HI1257	1329334	1330392	HI1413	1503610	1504026

HI1414	1504094	1502787	HI1526	1588628	1589692	HI1669	1732543	1731909
HI1415	1505280	1504099	HI1527	1589781	1590284	HI1670	1733332	1732556
HI1417	1506471	1505953	HI1528	1590287	1592155	HI1671	1733482	1733363
HI1418	1506880	1506602	HI1529	1592772	1593659	HI1672	1733919	1733539
HI1419	1507067	1506795	HI1530	1593826	1593975	HI1673	1735404	1733938
HI1421	1507987	1507634	HI1540	1605903	1606442	HI1675	1737711	1737589
HI1422	1508392	1508327	HI1541	1606426	1607595	HI1677	1738407	1739654
HI1423	1509030	1508428	HI1542	1607568	1607912	HI1678	1739641	1742283
HI1424	1509352	1509648	HI1548	1613326	1613877	HI1683	1745073	1745741
HI1425	1509648	1509938	HI1549	1614482	1613931	HI1685	1747304	1747843
HI1426	1510250	1509975	HI1551	1616455	1615214	HI1686	1750100	1747947
HI1427	1510403	1510975	HI1552	1616740	1617159	HI1687	1750833	1750171
HI1428	1511264	1511545	HI1554	1619807	1618560	HI1689	1752090	1753040
HI1431	1513776	1514795	HI1558	1622639	1621995	HI1690	1753041	1753619
HI1432	1514998	1515831	HI1561	1626292	1625114	HI1693	1757163	1757783
HI1439	1521750	1522223	HI1564	1628971	1628171	HI1694	1757788	1758492
HI1440	1522224	1525568	HI1566	1630319	1629852	HI1707	1770253	1770993
HI1441	1525569	1525820	HI1568	1631692	1631537	HI1709	1774757	1773684
HI1443	1526752	1528626	HI1569	1632481	1631948	HI1710	1775859	1774744
HI1450	1533358	1533038	HI1570	1632603	1632517	HI1715	1782227	1781865
HI1454	1536172	1536492	HI1572	1633105	1633257	HI1716	1782482	1782345
HI1455	1536633	1536668	HI1575	1636870	1636721	HI1720	1786560	1785523
HI1456	1537150	1536566	HI1576	1637376	1636870	HI1721	1786631	1787176
HI1458	1538541	1537903	HI1577	1637498	1637439	HI1723	1788842	1788747
HI1460	1540315	1539812	HI1586	1647922	1647857	HI1724	1789761	1788979
HI1462	1541101	1541340	HI1587	1648198	1648028	HI1726	1792471	1793034
HI1468	1547394	1546060	HI1588	1648605	1648189	HI1727	1793205	1793852
HI1474	1554422	1554078	HI1592	1654749	1653193	HI1729	1794860	1795201
HI1477	1557241	1556189	HI1596	1659183	1657846	HI1730	1795161	1795556
HI1481	1560071	1559355	HI1597	1659861	1659247	HI1736	1803407	1802481
HI1482	1560378	1560563	HI1599	1661605	1661453	HI1737	1804045	1803407
HI1484	1562720	1562989	HI1600	1662311	1661643	HI1742	1813528	1813298

HI1486	1563395	1562928	HI1601	1662648	1662328	HI1743	1813960	1813634
HI1487	1564353	1564667	HI1604	1665779	1664724	HI1744	1814691	1813960
HI1489	1565191	1565349	HI1605	1666807	1666094			
HI1490	1565824	1566042	HI1606	1667750	1666800			
HI1491	1566045	1566215	HI1607	1668067	1667783			
HI1492	1566221	1566778	HI1608	1668561	1668109			
HI1494	1567509	1568060	HI1609	1668769	1669446			
HI1495	1568255	1568467	HI1611	1670802	1671410			
HI1497	1568697	1569200	HI1613	1672733	1673359			
HI1498	1569285	1569566	HI1614	1673350	1674312			
HI1500	1569836	1570093	HI1618	1678855	1677464			
HI1501	1570093	1570344	HI1626	1686816	1686316			
HI1502	1570465	1570689	HI1627	1687436	1686819			
HI1503	1570599	1571015	HI1628	1687921	1687439			
HI1504	1571343	1571909	HI1630	1688617	1687937			
HI1505	1571912	1573435	HI1631	1689671	1689177			
HI1506	1573450	1575009	HI1632	1690500	1690847			
HI1507	1575103	1576344	HI1633	1690388	1689675			
HI1510	1578223	1579146	HI1634	1690881	1691282			
HI1511	1579232	1579486	HI1637	1693111	1692542			
HI1512	1579501	1579614	HI1643	1702285	1700876			
HI1513	1579620	1580042	HI1649	1707768	1708781			
HI1514	1580012	1580593	HI1653	1711982	1712854			
HI1515	1580609	1580797	HI1654	1712909	1713433			
HI1516	1580800	1582260	HI1656	1715939	1716046			
HI1517	1582273	1582626	HI1657	1716442	1716167			
HI1518	1582642	1583022	HI1658	1717744	1717196			
HI1519	1583106	1584998	HI1659	1718225	1717860			
HI1520	1584526	1584371	HI1660	1720257	1719409			
HI1523	1587316	1587624	HI1661	1720329	1722053			
HI1524	1587664	1588209	HI1662	1722056	1722412			
HI1525	1588221	1588625	HI1663	1722428	1723010			

**Table 3 Whole Genome Sequencing Strategy**

Stage	Description
Random small insert and large insert library construction	Randomly sheared genomic DNA on the order of 2 kb and 15-20 kb respectively
Library Plating	Verify random nature of library and maximize random selection of small insert and large insert clones for template production
High-throughput DNA sequencing	Sequence sufficient number of sequence fragments from both ends for 6X coverage
Assembly	Assemble random sequence fragments and identify repeat regions
Gap closure	Order all contigs (fingerprints, peptide links, lambda clones, PCR) and provide templates for closure
a. Physical gaps	Complete the genome sequence by primer walking
b. Sequence gaps	Visual inspection and resolution of sequence ambiguities, including frameshifts
Editing	Identification and description of all predicted coding regions (putative identifications, starts and stops, role assignments, operons, regulatory regions)
Annotation	

**Table 4** The theory of shotgun sequencing follows from the application of the equation for the Poisson distribution  $p_x = m^x e^{-m}/x!$  where  $x$  is the number of occurrences of an event and  $m$  is the mean number of occurrences. The numbers below predict the assembly of a 1.9 Mb genome with an average sequence fragment size of 460 bp.

N	% unsequenced	bp unsequenced	DS Gaps	Avg. Gap Length
250	94.44	1794304	236	7600
500	89.18	1694487	446	3800
1,000	79.54	1511204	795	1900
2,000	63.26	1201967	1265	950
3,000	50.32	956009	1509	633
5,000	31.83	604785	1592	380
10,000	10.13	192508	1013	190
15,000	3.23	61277	484	127
20,000	1.03	19505	205	95
25,000	0.33	6209	82	76
30,000	0.10	1976	31	63
50,000	0.00	20	1	38



Table 5 Summary of features of whole genome sequencing of *H. influenzae* Rd

Description	Number
Double stranded templates	19,687
Forward sequencing reactions (M13-21 primer)	19,346
# Successful (%)	16,240 (84%)
Average edited read length	485 bp
Reverse sequencing reactions (M13RP1 primer)	9297
# Successful (%)	7,744 (83%)
Average edited read length	444 bp
Sequence fragments in random assembly	24,304
Total # of base pairs	11,631,485
# of contigs	140
Physical gap closure	42
PCR	37
Southern analysis	15
Lambda clones	23
Peptide links	2
Terminator sequencing reactions*	3,102
# Successful (%)	2,024 (65%)
Average edited read length	375 bp
Genome Size	1,830,121 bp
# of N's in sequence (%)	188 (0.01%)
Coordinates of proposed origin of replication	602,483-602,764
G/C content	38%
# of rRNA	6
rmA, rrnC, rrnD (spacer region)	723 bp
rmB, rmE, rmF (spacer region)	478 bp
# of tRNA genes identified	54

Number of Predicted Coding Regions	1,749
# Unassigned role (%)	724 (41%)
No database match	384
Match hypothetical proteins	340
# Assigned role (%)	1025 (59%)
Amino acid metabolism	71 (6.9%)
Fatty acid/phospholipid metabolism	24 (2.3%)
Biosynthesis of cofactors, prosthetic groups, and carriers	54 (5.3%)
Purines, pyrimidines, nucleosides, nucleotides	54 (5.3%)
Central intermediary metabolism	31 (3.0%)
Energy metabolism	99 (9.7%)
Cell envelope	82 (8.0%)
Regulatory functions	63 (6.1%)
Replication	88 (8.6%)
Transcription	27 (2.5%)
Translation	146 (14.2%)
Transport/binding proteins	145 (14.1%)
Cellular processes	42 (4.1%)
Other	99 (9.7%)

\* Includes gap closure, walks on rRNA repeats, and random end-sequencing of lambda clones for assembly confirmation

Table 6 Two component systems in *H. influenzae* Rd

ID	Location	Best Match	%ID	%Sim	Length (bp)
<b>Sensors:</b>					
HI0221	239,378	<i>arcB</i> (E. coli)	39.5	63.9	200
HI0269	299,541	<i>narQ</i> (E. coli)	38.1	68.0	562
HI1713	1,781,143	<i>basS</i> (E. coli)	27.7	51.5	250
HI1381	1,475,017	<i>phoR</i> (E. coli)	38.1	61.6	280
<b>Regulators:</b>					
HI0728	777,934	<i>narP</i> (E. coli)	59.3	77.0	209
HI0839	887,011	<i>cpxR</i> (E. coli)	51.9	73.0	229
HI0886	936,624	<i>arcA</i> (E. coli)	77.2	87.8	236
HI1382	1,475,502	<i>phoB</i> (E. coli)	52.9	71.4	228
HI1714	1,781,799	<i>basR</i> (E. coli)	43.5	59.3	219

## (1) GENERAL INFORMATION:

- (i) APPLICANTS: Human Genome Sciences, Inc.;  
Johns Hopkins University
- (ii) TITLE OF INVENTION: The Nucleotide Sequence of  
the Haemophilus influenzae Rd Genome, Fragments  
Thereof, and Uses Thereof
- (iii) NUMBER OF SEQUENCES: 1
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Sterne, Kessler, Goldstein & Fox, P.L.L.C.
  - (B) STREET: 1100 New York Avenue, N.W.
  - (C) CITY: Washington
  - (D) STATE: DC
  - (E) COUNTRY: USA
  - (F) ZIP: 20005-3934
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: 3 1/2 inch diskette
  - (B) COMPUTER: Dell Pentium
  - (C) OPERATING SYSTEM: MS DOS v6.22
  - (D) SOFTWARE: ASCII Text
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: (Not Yet Assigned)
  - (B) FILING DATE: (Herewith)
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/476,102
  - (B) FILING DATE: 07-JUN-1995
- (viii) PRIOR APPLICATION DATA:
  - (A) APPLICATION NUMBER: US 08/487,429
  - (B) FILING DATE: 07-JUN-1995

## (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/426,787

(B) FILING DATE: 21-APR-1995

## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1830121 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCCGCGGTG CATCACAAAA CATCATTTCCA TCTTCAACAG GTGCAGCGAA AGCAGTAGGT	60
AAAGTATTAC CTGCATTAAA CGGTAAATTA ACTGGTATGG CTTTCCGTGT TCCAACGCCA	120
AACGTATCTG TtGTTGATTT AACAGTTAAT CTTGAAAAAC CAGCTTCTTA TGATGCAATC	180
AAACAAGCAA TCAAAGATGC AGCGGAAGGT AAAACGTTCA ATGGCGAATT AAAAGGCGTA	240
TTAGGTTACA CTGAAGATGC TGTTGTTTCT ACTGACTTCA ACGGTTGTGC TTAACTTCT	300
GTATTTGATG CAGACGCTGG TATCGCATTa ACTGATTCTT TCGTTAAATT GGTATCTTGG	360
TACGATAACG AAACGGGTTA CTCAAACAAA GTATTAGACT TAGTAGCTCA TATCTACAAC	420
TACAAAGGCT AATTAAACT TTGAAAAAAT TAACCGCTCT TCGGAGCGTT TTTTATTATC	480
TAGAATTTAA TTTACGCTCT AAAAATGAAC AAGGGATCAC TAAAAATAAT TTAAACAAT	540
CAATATTCTT CTAGCTTTTA TTCCTATTTA AGATTATATT AGCGCACAAAC TGTTGCTCA	600
ATGAAAATCA AAAATAGGGT TAATATGAAT CTCGATCTCC ATTTTGTTCA TCGTATTCAA	660
CAACAAGCCA AAACGCTAC AAATATGACC GCACTTCGCT ATAAAGAACA CGGCTTGTGG	720
CGAGATATCT CTTGGAAAAA CTTTCAAGAG CAACTCAATC AACTTTCTCG AGCATTGCTT	780
GCTCACAATA TTGACGTACA AGATAAAATC GCCATTTTGT CCCATAATAT GGAACGTTGG	840
ACAATCGTTG ACATTGCGAC CTTACAAATT CGAGCAATCA CAGTGCCTAT TTACGCAACC	900
AATACAGCCC AGCAAGCAGA ATTTATCCTA AATCACGCCG ATGTAAAAAT TCTCTTCGTC	960
GGCGATCAAG AGCAATACGA TCAAACATTG GAAATTGCTC ATCATTGTCC AAAATTACAA	1020
AAAATTGTAG CAATGAAATC CACCATTCAA TTACAACAAG ATCCTCTTTC TTGCACTTGG	1080

GAAAGTTTTA	TTAAAACAGG	TTCAAACGCC	CAACAAGATG	AACTAACCCA	ACGCTTAAAC	1140
CAAAAACAAT	TATCGGATTT	ATTTACGATT	ATTTATACCT	CAGGCACAAC	GGGAGAGCCT	1200
AAAGGTGTCA	TGTTAGATTA	CGCTAATCTC	GCTCACCAAT	TAGAAACACA	TGATCTTTCA	1260
CTTAATGTGA	CAGATCAGGA	TATTTCACTT	TCTTTTTTAC	CATTCTCTCA	TATTTTTTGAA	1320
CGGGCATGGG	CGGCTTATAT	TCTTCATAGA	GGCGCAATAC	TTTGCTATTT	AGAAGACACT	1380
AATCAAGTGC	GGTCAGCTTT	AACGGAAATT	CGCCCAACTT	TAATGTGCGC	CGTACCACGT	1440
TTTTACGAAA	AAATTTATGC	TGCCGTATTG	GATAAAGTTC	AAAAAGCCCC	AAAACCTTCGC	1500
CAAATTATGT	TTCATTGGGC	AATTTCCGTG	GGACAAAAAT	ATTTTGATCT	ACGTGCGAAT	1560
AACAAAGCTA	TTCCGTTCTT	ATTGAAGAAA	CAATTTGCTT	TAGCAGATAA	ATTAGTGCTC	1620
TCAAAACTTC	GTCAATTATT	GGGGGGACGA	ATAAAAATGA	TGCCTTGCGG	AGGAGCAAAA	1680
TTAGAACCGG	CTATTGGGCT	ATTTTTCCAT	GCTATTGGTA	TCAACATCAA	ATTAGGCTAT	1740
GGTATGACAG	AAACAACGTC	AACCGTTTCT	TGCTGGCATG	ATTTCCAATT	TAACCCAAAT	1800
TCAATCGGCA	CACTGATGCC	AAAAGCGGAA	GTGAAAATTG	GGGAAAATAA	TGAAATCCTT	1860
GTGCGTGGCG	GAATGGTGAT	GAAAGGCTAT	TACAAGAAGC	CAGAAGAAAC	GGCTCAAGCC	1920
TTCAACGAAG	ATGGTTTCTT	AAAAACTGGC	GATGCAGGAG	AATTTGACGA	ACAAGGCAAT	1980
TTATTTATTA	CCGATCGTAT	CAAAGAATTA	ATGAAAACCT	CAAACGGCAA	ATATATCGCA	2040
CCACAATATA	TCGAAAGCAA	AATCGGTAAA	GATAAATTTA	TCGAACAAAT	TGCGATCATC	2100
GCTGATGCGr	AAAAATATGT	ATCCGCGCTT	ATTGTGCCTT	GCTTTGATAG	TTTGAAGAA	2160
TACGCTAAAC	AGCTCAATAT	TAAATATCAT	GACCGTTTAG	AACTACTAAA	AAATTCTGAC	2220
ATTCTGAAAA	TGTTTGAGCA	TCGTATTAAT	GCGGTGCAAA	AAGAATTGGC	TCATTTGAG	2280
CAAGTAAAAA	AATTCACGTT	ACTTTCTCAA	GCATTGAGCA	TTAAATTAGG	CGAAATTACA	2340
CCAACATTAA	AATTACGTAG	AAAAGTGATT	TTAGAACGTT	ATCGCAAGCA	AATTGAAGCA	2400
ATGTATCATT	CACAAGAAGC	ATAGCTAAAA	GGCTCTCAAT	TGAGAGCCTT	TCTTTTCTAA	2460
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CACATGTTCT	AGCATATCCA	GATCATTAAT	ATTATCGCCA	AATGCAATCA	CTTCATTAGT	2640
TTGTACGCCA	AAATAATCTT	CCAAAAAACG	CACCGCACTT	CCTTTGGTTG	CACTCTTGTTG	2700
CATTACCTCT	AAAAAATTAG	CGTGGGAACG	ACAAATACTT	AAATGTGGAA	ATTTTTCTCT	2760

TAAAAGAACT TCAATCTCAA TGATTTCTTC TGCTTCCCCA ATAATTTGAA TTTTATGTGG	2820
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AATCACCCAT TTATTTTCTA CATCGCGCGC ATGGCAATCA TTATTTGTAT AATAATTCAC	2940
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TAACAATGGC AACTCCACCT CATCGGGGCA TTCAAATGGA AATGAAAGCA CGTTAGTCGG	3540
GCGATCTTTT CCACGATAAG TTAAATTTAA TTCATGGCTT TCGGCTTCAT CCACAATACG	3600
CACCGTCATT TCGACTTCAT TGCCCTCAGG CTGAACAGCA CCTGTTGCCC ACTGCACAAT	3660
CTGCTCTTCT GTTGGCAAAC CCTCTATATT TTCTGTGGCA ATTTGCAAAT CAACCAATAC	3720
ACTTCCCATT CTATTCTCCT AAATTCTTTT CAATTTCTAA CCGCACTTTT GCACTTATTG	3780
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AATTTACTCT ATTTTAAACG TACAAAGAGA CAAATCACGC TATTTCTTA ATCAACACAA	3960
AACCTCAAAT CTCTCGCTCT TGTTGAGGT TAATTGTGAA GTCGTAGATG TCCGCTATAA	4020
ATATTTCTT TTCTTCCCT AGCAAAACCA ATTAAAGTCA AATTATGTTT TTCTGCCATT	4080
GTTACTGCTA AATCCGTTGC GGCAGAAATC GTGACTAACA TTTCCACCCC GCAAGCCACT	4140
GTTTTTTGCA CCATTTTATA ACTCGCTCGA CTGGAGGCTA AAATAAAACC TCTAGGTTTA	4200
CCCGATTTTG CGTGCCAACC AAGCAATTTA TCTAATGCAA CGTGGCGACC CACATCTTCG	4260
TAAATAGCTA ACATACTTCC ATATAAATCG AAAAATGCAC AAGCATGGGT AGCACCAGTT	4320
TTACATCCAA GCAATTGATT TTTTGAAGA TCAATAAGAC AACTATCTAG CAAATTTAAA	4380
TCAAATTGAA AAGTACAGTC TAATTTCCGA AAAGTTTTAT ATACTTGATT CAATTGTTCT	4440

GTACCACAAA TACCACAACC AGTACGCCCC GTAAGATTAC GACGATGCTC TTTTAATGCC	4500
ATAAATTTAC GGCTGGAAAG TTCAATTTGC ACTTCAATAC CATTGCAAAC TTCTACAACA	4560
TCAATACCAT AAATATCTTC TGGTTTATTG ATAATTCCCT CTGTTAGAGA AAAGCCTAAA	4620
GsAAAATCCT CTAAATTGTT TGGGGAACAC ATCATAACTG CGTGAGAAAT GCCGTTATAT	4680
ACCAAAGAAA CTGGTATCTC AACAGCCAGT ATGTCCTCTT TTTGTTGCAA AAGTGCGGTC	4740
AATTTTnCTG TATTTTTTGT CTTTCTAAAA AAGTTGATAG TTTGTCGAAT CCAGCACTGC	4800
ATTTATTACC AGTCCATTAG TTAAATGTAA AAAGTCTGTT AAATTATTTT TATATTTATG	4860
ATCTAGATCA CAATATTTGT TGAAGCTAAT GTCATTTGAT TATGGATTTA TCGTTAAAAA	4920
ATCGTTACTC TAGCCATCCA ATAAATCAAC GTTTTTACGA TGATTTATAT ACTAGAGAGT	4980
ATTATTTTAA CGAAATCAAC ATAATCTAGC TACTAAACGA ATTTTATTAC TATCTATAAT	5040
ATCTCAACAA CTTGTTGTTG AAAGGAGTGA TTATGCAGGT CTCAAGAAGA AAATTCTTCA	5100
AGATCTGTGC AGGAGGTATG GCGGGAACGT CAGCTGCAAT GTTGGGCTTT GCTCCAGCAA	5160
ACGTATTAGC TGCGCCACGC GAATATAAAT TATTACGCGC GTTTGAATCC CGTAACACCT	5220
GTACATATTG CGCTGTAAGT TGCGGTATGT TGTATATAG CACAGGCAAA CCTTACAATT	5280
CATTAAGCAG CCATACTGGC ACAAATACTC GTTCAAACT CTTTCATATT GAGGGTGATC	5340
CAGATCATCC AGTCAGTCGT GGTGCGCTTT GCCCGAAAGG TGCTGGCTCA CTCGATTATG	5400
TCAATAGTGA AAGCCGTTCT TTATATCCTC AATATCGTGC GCCAGGTTCT GATAAATGGG	5460
AACGAATTTT TTGGAAAGAT GCCATTAAAC GTATTGCTCG TTTAATGAAA GATGACCGAG	5520
ATGCCAACTT TGTTGAAAAA GATTCAAATG GAAAAACGGT TAATCGTTGG GCAACGACAG	5580
GAATTATGAC TGCATCAGCA ATGAGCAATG AAGCTGCGTT ATTAACACAA AAGTGGATTA	5640
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ACGCGCTGCT	CTGTGCCAAG	CATACGACGG	CTAAATTGTG	CCGCTTGTTG	ATTAATACGC	19140
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TGTGCAATCA	AATTCATAGT	TTGCTCAAAA	TCTTCCGCCG	TTTACCAGG	GAAACCAACA	19320
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TCATCGGTAA	ATTCTATTGG	ATGACTGGTT	GTGAACGTAA	ACGGTCAATA	CCATCAATTG	19560



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GCGCAATTTT	AAATAGCACA	TCATCTACAG	GACGGCTAAC	TTCTTCTCCA	CGAGTATAAG	19740
GCACAACACA	AAAAGTACAG	TATTTATTAC	AGCCTTCCAT	AATGGAAACA	AATGCCGTTG	19800
GGCCTTCTGC	GCGAGGTTCT	GGTAAGCGGT	CAAATTTCTC	AATTTCAGGG	AAACTTACGT	19860
CTACGACGGA	ACTTTTTTCCA	CCACGAATTT	GATTAATCAT	TTCAGGCAAG	CGATGCAAAG	19920
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GCGCCTGTTA	CATGTCCGAC	TGTTGCAAAT	ACGGGTACCA	TTGCAGCTAC	ATATGCACCG	20940
CCTGATGCGA	ATAAATAACG	TACAGAGACA	CTTAAGGTAA	GAATCACTAT	TAACGCCATC	21000
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GAATACCGAA	CTTAGTCCTT	TTAATGAAAG	TGATTTAACG	TTTGAATTTA	TTGCTTACGC	84000
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GCCTCAATTT	TTGCCACTTC	ATCATCGTTA	GAACCTGCTT	GTAAGAATTT	CTCTGGTTCT	84180
TGTTGAAGTA	ATCCTAAAAAT	CGCCCCCAAT	TCACGCAAAC	GTGCAGCAAG	TCCATTAGCT	84240
TTCTCAACAT	CTTCTGTTTT	TAACCTATTA	ATTTACGCTG	CCATTTTCGAA	TAAAACAGAA	84300
AGTGCATTTG	GCGTATTAAA	ATCATCATCC	ATTGCTTCAC	GGAAAGTTGC	AACAAAATTT	84360
TCTCCGCCAA	AAGCAACCGC	ACTTTGATCT	GTTCCGCGTA	AAGCCGTGTA	TAAACGTTCC	84420
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GCCGTTAATA	AGAAATAACG	CACCGCTTCT	GCATTGTAGT	GGTTTAATAC	ATCACGAATT	84540
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-77.56-

**SUBSTITUTE SHEET (RULE 26)**

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